Influence of Turbidity on Social Structure in Guppies, *Poecilia reticulata*

DISSERTATION

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von
Diplom-Biologin Karoline Borner

Präsident der Humboldt-Universität zu Berlin

Prof. Dr. Jan-Hendrik Olbertz

Dekan der Lebenswissenschaftlichen Fakultät

Prof. Dr. Richard Lucius

Gutachter:

1. Prof. Dr. Jens Krause
2. PD. Dr. Thomas Mehner
3. Dr. Daniel W. Franks

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Content

Abstract

Most species have been subjected to environmental changes during their evolutionary history. However, due to human activity, environmental changes are currently occurring at higher speeds and on a greater scale, presenting new challenges for many species. Pollution, as a major type of human-induced environmental change, may not only affect physiology but also behaviour, thereby affecting population dynamics and consequently biodiversity. The topic of my dissertation is the effect of turbidity from quarrying on the behaviour and social association pattern of the guppy (*Poecilia reticulata*). Turbidity impairs the fish's ability to use visual cues during social interactions, which in turn helps increases efficiency of foraging and avoiding predators. I investigated the initial response of guppies to turbidity and subsequently tested whether turbidity-experienced vs turbidity-inexperienced populations differ in their social association patterns and how they cope with ecological challenges. Both lab-reared and wild-caught guppies that were inexperienced with turbidity reduced social associations in turbid water in contrast to turbidity-experienced fish. A Markov chain analysis revealed that lab-reared guppies increased associations with particular neighbours. Similarly, wild-caught guppies maintained the number of initiated associations under turbid conditions. The increase in non-initiated associations suggests a stronger connectivity within the shoal, leading to higher information transmission in a poor visual environment. Additional results showed that this altered social structure enabled turbidity-experienced fish to maintain the frequency of mating attempts in turbidity and to avoid predation risk. This suggests that guppies have the ability to adjust to turbidity, but with major changes in their social structure, which might have an impact on population dynamics.

Keywords: turbidity, guppy, *Poecilia reticulata*, social association, shoaling, predation risk, predator avoidance, social behaviour

Zusammenfassung

Umweltveränderungen kommen natürlicherweise vor und viele Spezies waren im Laufe ihrer Evolutionsgeschichte davon betroffen. Durch die Aktivitäten des Menschen jedoch finden diese in höherer Geschwindigkeit und größerem Umfang statt und stellen so für viele Spezies eine neue Herausforderung dar. Einen großen Einfluss auf die Umwelt nimmt der Mensch durch Verschmutzung, welche zu Veränderungen der Physiologie der Organismen und deren Verhalten führen und damit Einfluss auf die Populationsdynamik und letztendlich auf die Biodiversität haben kann.

In meiner Dissertation untersuchte ich den Einfluss durch Bergbau ausgelöster Trübung auf das Verhalten und die soziale Struktur des Guppys. Er nutzt soziale Interaktionen für eine höhere Effizienz bei der Nahrungssuche und Räubervermeidung. Die Nutzung seines dafür eingesetzten Sehsinns ist bei Trübung stark eingeschränkt. Ich untersuchte die Reaktion Trübung unerfahrener Fische aus Labor und Feld auf Trübung. Es zeigte sich, dass beide ihre sozialen Interaktionen in trübem Wasser verringerten. Eine zusätzliche Markow-Ketten-Analyse ergab aber auch, dass Laborfische Kontakte zu bestimmten Individuen der Gruppe verstärkten und Feldfische ihre initiierten Kontakte behielten. Anschließend studierte ich den Unterschied der sozialen Struktur Trübung erfahrener und - unerfahrener Fische. Trübung erfahrene Fische erhöhten die Gesamtzahl der Interaktionen, reduzierten jedoch die Anzahl der initiierten Kontakte im Gegensatz zu unerfahrenen Fischen.

Diese Strukturänderung, vermute ich, erhöht den Zusammenhalt und damit den Informationsfluss im Schwarm. Die Ergebnisse von Folgeversuchen, nämlich der Erhalt der Paarungsanzahl und die effektivere Vermeidung einer Räuberattrappe bei Trübung erfahrenen Fischen, unterstützen diese Vermutung. Die Arbeit zeigt, dass Guppys in der Lage sind, sich durch Änderung Ihrer sozialen Struktur an trübe Verhältnisse anzupassen. Dies könnte auch Einfluss auf ihre Populationsstruktur haben.

Schlagwörter: Guppy, Trübung, Schwarmverhalten, Sozialverhalten, Räubervermeidung, *Poecilia reticulata*

Thesis outline

This thesis contains a summarizing chapter and three independent manuscripts that are either published (Chapter I), submitted to a peer-reviewed journal (Chapter II) or a draft of a manuscript (Chapter III). Therefore, every chapter stands alone including an introduction, methods, results and discussion section. In the summarizing chapter, I provide an overview of the three chapters including the context and the aim of the thesis and a general discussion that connects the findings of the three chapters and provides further perspectives.

Author contributions to the chapters:

Chapter I:

Turbidity affects social dynamics in Trinidadian guppies

Karoline Borner, Stefan Krause, Thomas Mehner, Silva Uusi-Heikkilä, Indar W. Ramnarine, and Jens Krause

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In the first chapter, I conceived the experiment in discussion with Thomas Mehner and Silva Uusi-Heikkilä. I carried out the experiment and drafted the manuscript, which I discussed with the scientific writing course at IGB. The statistical analysis (GLMMs) I conducted in discussion with Thomas Mehner. The Marcov chain analysis and its description in the manuscript have been done by Stefan Krause and Jens Krause.

Chapter II:

Turbidity-induced changes in shoal structure of guppies (*Poecilia reticulata*) reflect origin-specific experience with turbidity

Karoline Borner, Jens Krause, Indar W. Ramnarine, and Thomas Mehner

Manuscript

In the second chapter, I conceived the experiment in discussion with Thomas Mehner and Jens Krause. Further, I applied for and received funding for the research trip to Trinidad. I conducted the experiment and the statistical analysis, which I discussed with Thomas Mehner and other scientists of a GLMM discussion group at IGB and drafted the manuscript.

Chapter III:

Experience-dependent behavioural responses to ecological challenges in turbidity
Karoline Borner, Thomas Mehner, Indar W. Ramnarine, and Jens Krause

Draft of manuscript

In the third chapter, I conceived the experiment in discussion with Jens Krause and Thomas Mehner and conducted it. The statistical analysis I carried out in discussion with Thomas Mehner and drafted the manuscript.

General introduction

Environments naturally vary over time and space but changes are currently occurring worldwide on a larger scale and at a higher speed due to human activities. These changes strongly impact species living in affected habitats and put organisms into novel environmental conditions that arise more rapidly than organisms have experienced in their evolutionary past (Sih et al. 2011). Numerous studies have been done on the behavioural response of several species to human-induced environmental changes (Tuomainen and Candolin 2011) and it is proposed that phenotypic plasticity (Hendry et al. 2008), more precisely, behavioural plasticity plays an important role in an animal's response (Sih 2013). Interestingly, human-induced environmental changes have differing effects on different species. While some species respond poorly behaviourally to environmental change, in extreme cases leading to species decline, other species cope well or even thrive. Conceptual frameworks have been suggested to better understand variation in behavioural response Sih (2013) but these frameworks focus on variation of behavioural responses at the individual level. So far, however, group responses to human-induced environmental changes are relatively unexplored and not included in Sih's concepts (Sih 2013).

The latter is an important point since group living is a common phenomenon in the animal kingdom and in many species different grouping types have evolved over time (Krause and Ruxton 2002). Birds and ungulates, for example accumulate in flocks and herds for migration. Insects build highly complex colonies with division of labour and many fish species live in shoals at least part of their lives (Shaw 1978). The composition of groups is commonly non-random (Krause and Ruxton 2002). Fish, for example, prefer shoal mates of their own species or even kin, individuals with similar size and appearance to themselves and they may also consider the parasite load in selecting shoal mates (Pitcher and Parrish 1993). Being in a group also serves multiple functions. In response to a predator threat, grouping is a beneficial reaction facilitating increased anti-predator vigilance, predator confusion or risk dilution (Krause and Ruxton 2002, Krause et al. 2011). It may further provide access to mates or facilitate hydrodynamic efficiency in shoaling fish. Group living may also increase the chance of finding profitable food patches (Pitcher et al. 1982, Vollset and Bailey 2011) and facilitates social information transfer and social learning (Fernández-Juricic and Kacelnik 2004, Webster and Laland 2011). On the other hand, if a resource, such as food or mates, is scarce, competition might be higher and it might be more benefi-

cial for an individual to leave the group (Magurran and Pitcher 1983, Croft et al. 2003a, Hoare and Krause 2003). Therefore, each individual decides continuously whether to join, stay or leave a shoal. To appraise the costs and benefits of being in a group, individuals need to continuously sample general information to remain up to date about the current environmental conditions (Inglis et al. 2001, Warburton 2006). Next to personal information sampling, which may be energetically costly if the sampling area is big or risky (if predators are nearby), individuals can acquire information from other individuals. This is accomplished through visual, chemical and acoustic communication (Zahavi and Zahavi 1999, Sluijs et al. 2010). Via particular cues, information is transmitted among shoal mates. Visual information transmission, for example, requires close contact facilitated by shoaling, where information can be visually transmitted by individual fish observing active signalling or reactions of shoal mates to external stimuli (Reader et al. 2003, Hoare and Krause 2003, Webster and Laland 2011).

Information transmission can be influenced if cue discrimination is disturbed. One major type of human-induced environmental change is pollution. It can mask cues or reduce sensitivity of the receiver and hence influence communication. Several studies demonstrate that disturbance due to pollution may have a strong impact on communication processes in animals. Mate preferences, for example, can be changed because of chemical or visual disruption (Fisher et al. 2006, Candolin et al. 2007), noise pollution can alter calling rates or song frequencies (Slabbekoorn and Peet 2003, Bee and Swanson 2007) and mate choice can be impaired due to poor visibility caused by turbidity (Seehausen et al. 1997). Although these studies involve social interactions, the influence of impaired cue mediation on social structure is largely unknown.

Turbidity in freshwater bodies is a phenomenon whose incidence is increasing worldwide; often generated by human activities that disturb land, such as construction, quarrying and agriculture (Conley et al 2009, Maya et al 2012, Sluijs 2011). Due to intense use of fertilizers in agriculture, nutrient run-off can result in eutrophication from an increase of algae, microbe and phytoplankton growth in the water body. Construction, mining and quarrying are closely linked to deforestation. These types of land usage release clay, silt and sand, which are washed into the rivers and lakes with every rainfall, leading to high turbidity levels. Turbidity is the level of cloudiness of water caused suspended particles scattering light. High levels of suspended particles reduce the amount of light that passes through the water, which can inhibit the growth of submerged aquatic plants and conse-

quently affects the habitat and food abundance of species that depend on them. In particular, algae and phytoplankton can shift the wavelength of the light altering the optical properties of the habitat (Utne-Palm 2002). Aquatic organisms that depend on visual communication for social interactions such as shoaling fish may be affected by turbidity due to the disruption of visual information transmission. A changed shoaling behaviour may impact foraging efficiency and the effect of antipredator behaviour of individual fish (Gregory and Northcote 1993, Lehtiniemi et al. 2005). It remains a largely unanswered question as to how individuals cope with rapidly appearing changes and cope with ecological challenges such as food finding or predator defence under human-induced environmental changes.

The Trinidadian guppy, like many other fish species, has well developed eyes (Evans et al. 2011), suggesting that environmental orientation and communication is at least partially vision based (Guthrie and Muntz 1993). However, some areas of its habitat are strongly affected by regularly occurring high turbidity. During the last two decades quarries have been established in some areas in the Northern Range of Trinidad, resulting in strong environmental changes including highly disturbed rainforest, deforestation and large quantities of clay entering the streams (Agard et al. 2004). Above the quarries, the rivers are largely undisturbed and the water remains largely clear with about 5 nephelometric turbidity units (NTU). In contrast, the river sections below the quarries are disturbed by regularly increasing turbidity. With every rainfall, run-off from the quarries is washed into the rivers leading to high turbidity in guppy habitat. Guppies are known for particular social and anti-predator behaviours (Magurran 2005). They spend a lot of their time in highly dynamic social groups, with populations from different rivers having a wide range in their level of sociability. In eight different rivers (Magurran and Seghers 1991) it was found that the percentage of individual fish being solitary ranged from 0.4% to 84%. In addition (Croft et al. 2003b) found that individuals encounter conspecifics on average every 14 s which would provide opportunities for switching partners. These conditions lead to typical fission-fusion dynamics in guppy shoals (Wilson et al. 2014) that enable guppies to transfer information and to benefit from social interactions. There are several social behavioural differences between males and females. Females build stronger connections and establishing more stable social networks than males (Darden et al. 2009). Females are sexually receptive for only a few days and therefore focus on social contact with other females to try to avoid harassment from males (Magurran and Seghers 1994, Piyapong et al. 2009). In

contrast, males invest more in mating behaviours (Magurran and Seghers 1994) spending more time searching for mates and attempting to mate with fertile females (Croft et al. 2003a). In addition, males get much closer to the potential predator during predator inspection than females (Magurran 2005).

The guppy has been a classic model system for evolutionary and behavioural biology over the last 70 years (Houde 1997), primarily because different guppy populations live in close geographic proximity but experience very different environmental conditions (Magurran 2005) and therefore provide excellent field study sites. One can find high and low predation sites in close proximity, shaded and sun-drenched pools, deep and shallow pools and differing currents. In addition, different river systems occur, such as the Caroni drainage and the Oropouche drainage, providing different genetic lines. Guppies therefore provide an ideal system in which to investigate how environmental change, such as changes in water turbidity, may influence behavioural decisions, such as shoaling, foraging and anti-predator behaviours.

Aim of thesis

The aim of my thesis was to understand the impact of intermittent human-induced clay-turbidity on activity and association structure of guppy shoals in dependence of earlier experience with turbidity. In addition, I tested its influence on fish dealing with ecological challenges.

In the first chapter, I identified the initial behavioural responses of turbidity-inexperienced guppies to turbidity. Guppies spend most of their time shoaling with others, a (largely) vision mediated behaviour, to gain benefits associated with shoaling. I hypothesized that increased turbidity will alter the activity and conspecific association behaviour of the fish. I predicted that due to the reduced ability to use visual cues group size would decrease and activity levels would be altered.

In my second chapter I raised the question whether the observed changes in activity level, group size and association preferences for particular neighbours of guppies in clear and turbid water found in chapter I were merely a constraint to cope with turbidity or a behavioural response to changed visual environment. This can be tested in a comparison of behavioural response to turbidity of fish that had previous experience with turbidity and

ones without (i.e. naïve fish). There exist guppies in the Northern Range of Trinidad that are strongly affected by regular turbidity due to quarrying activities and hence are experienced with turbidity, while others are not affected by turbidity and are hence inexperienced with regular turbidity. I hypothesized that fish's previous experience with turbidity is associated with behavioural association differences. Experienced fish should maintain their shoal size to maintain shoaling benefits among shoal-mates and alter their activity level in order to counterbalance reduced contacts due to poor visibility in turbid water. Inexperienced fish should display a decrease of associations when exposed to turbid water conditions, as they had no previous experience with how to cope with this environmental change. I found behavioural and association differences in the response of experienced and inexperienced fish to turbidity.

This led me to develop the experiments described in Chapter III. I wanted to test whether those differences described in Chapter II would help populations handle ecologically relevant tasks differently in the event of increased turbidity. I exposed experienced and inexperienced fish to food and predator dummies, respectively as these factors are paramount ecological challenges, both in clear and turbid water. I hypothesized that experienced fish would respond appropriately to a food and predator item and I predicted that experienced fish should stay longer with the food and better avoid the predator in turbid water, whereas inexperienced fish should not differ in their response to both stimuli in turbid water and hence not cope well with these ecological challenges.

Main methods

Experiments described in Chapter I were performed on descendants from wild-caught Trinidadian guppy populations. These fish had lived under laboratory conditions for about 20 generations and thus had never experienced turbid water themselves. In total 100 fish in ten test groups with ten fish in each group were observed in turbid and clear water treatment in random order. Fish were individually marked with colour codes on the back and each fish was observed in random order with focal follow (see Wilson et al. 2014) where individual fish were followed over for 2 x 3 min and information about its neighbours and location in the test tank were collected every ten seconds. Data about the location of fish over time provided information about activity level and the number of neigh-

bours could be used to calculate group size. I used generalized linear mixed models (GLMM) to test whether water turbidity affected activity level and group size. Data about temporal association patterns of the fish were further used in a Markov chain model to capture the fission-fusion dynamics underlying the social interaction patterns of the fish. In the model, a focal fish can have two states, one state of being alone the other of being social. A focal fish can be social with different neighbours and hence the number of states of being social is exactly the number of potential neighbours of the focal fish. By regarding those states in a first-order Markov chain, the transition probabilities between these states can be estimated from the data points in our observations. Our model then predicts the distributions of the lengths of social contact phases (distinguishing between different nearest neighbours) and of being alone. The model also predicts the lengths of contact phases with the same neighbour. Tests of association preferences with particular neighbours can be made.

In my second chapter I described an experiment I developed to evaluate differences in the behavioural response of turbidity-experienced and turbidity-inexperienced guppies and used a more naturalistic set up than in the first experiment to copy the changing turbidity conditions observed in the field. I collected wild guppies that lived above (inexperienced fish) and below quarries (experienced fish) in the Arima River, Northern Range, Trinidad. Collection sites of experienced and inexperienced fish were located as close as possible to each other to limit any other ecological differences between populations. To mimic natural conditions I conducted the experiments in a sequence as it appears naturally in the field: first, clear water, then, short-term turbidity, as it appears when heavy rain starts and washes clay into the river, and lastly, long-term turbidity, which can be maintained for hours after rainfall. In total 200 fish with ten groups of ten fish from each origin, were exposed to each of the three different turbidity treatments. Fish were individually marked and I used focal follows of each fish in each treatment every 10 seconds for 2 min to collect data about its neighbours, its mating attempts and its location in the test tank. I used an observational method, rather than the Markov chain model, to identify association preferences and defined two different association states. Guppies may initiate association to particular neighbours with targeted swimming towards this fish and continuing movement with corresponding fish. All non-initiated associations are referred to as local associations. I tested with GLMM whether different water turbidity levels affected the activity

level, the time spent alone, the number of mating attempts and the number of initiated and local associations of experienced and inexperienced fish differently.

In the experiment described in my third chapter, I collected experienced and inexperienced wild guppies from the Arima and the Turure River, Northern Range, Trinidad. Again, collection sites for experienced and inexperienced fish from the same stream were as close as possible to each other. In total 400 fish, with ten groups of ten fish from each origin and stream were exposed to clear water treatment and long-term turbidity. In each treatment fish were exposed first to a food dummy and subsequently to a predator dummy. I used a focal follow procedure where I observed only one fish per group serving as an indicator for the whole group, which made the procedure of individual marking unnecessary. The focal fish of each group was observed in each turbidity treatment with each dummy over 6 minutes and every ten seconds data about its location were collected. With GLMMs I tested whether experienced and inexperienced fish spent differing frequencies close to the dummies depending on the turbidity level.

Main results

Altogether, there was strong evidence that turbidity influences association pattern in guppies (Chapters I and II). Lab-reared guppies with no turbidity experience decreased the number of social interactions under turbid conditions compared to social behaviour in clear water (Chapter I). Fish formed smaller groups and spent more time alone when in turbid water. Additionally, the Markov chain analysis provided further detail about the kind of associations that were affected and showed that fish spent longer periods with a particular neighbour. This means that lab-reared turbidity-inexperienced fish reduced the number of social interactions at cost of associations with non-particular neighbours. In addition, fish were found to be less active in turbid water.

Similar to the lab-reared guppies, wild-caught guppies with no turbidity experience decreased the number of social interactions under turbid conditions, compared to social behaviour in clear water (Chapter II). Inexperienced fish reduced social interactions and spent more time alone. More specifically, inexperienced fish reduced the number of local associations but maintained a similar number of initiated associations. We used two different methods to gather information about associations to particular neighbours as de-

scribed in the methods. Yet, we found similar results in lab-reared and wild-caught turbidity-inexperienced fish. This suggests that behavioural responses of turbidity-inexperienced lab-reared and wild-caught fish regarding turbidity may be comparable and potential conclusions may be transferable.

In contrast to the behavioural response of inexperienced fish, wild-caught fish with earlier experience with turbidity increased the number of social interactions under turbid conditions, compared to the number of social interactions in clear water (Chapter II). Turbidity-experienced fish exhibited larger group sizes and spent less time alone in turbid water but reduced initiated associations. Further, experienced fish re-established the number of mating attempts in long-term turbidity, which was not seen in inexperienced fish. Additionally, both experienced and inexperienced fish decreased activity under turbid conditions but experienced fish decreased activity further in long-term turbidity.

In the third chapter, I found evidence that earlier experience with turbidity changes the way in which individuals cope with ecological challenges in turbid water. While experienced fish avoided the stimulus area during the predator treatment, inexperienced fish displayed the opposite response spending slightly more time with the predator. Interestingly, there was no difference between experienced and inexperienced fish in the food treatment.

General discussion

The experimental results support my hypothesis that turbidity has a strong effect on social association patterns of guppies. Depending on earlier experience with turbidity, guppies responded differently to increasing turbidity and to ecological challenges. Experienced fish increased overall associations and handled predation risk more adaptively with being more cautious in turbidity compared to inexperienced fish. The observed difference in responses may have consequences for individual fitness and hence affect the viability and the distribution of populations.

Inexperienced fish responded with an overall decrease of social associations and thus reduced shoaling. Decreased shoaling may be a result of poor visibility and fish being impaired in visually detecting conspecifics. Previous investigations in fish detected sensory constraint leading to reduced association behaviour; males of the sailfin molly, *Poecilia*

latipinna, associated less with females under turbid conditions (Heubel and Schlupp 2006) and yellowtail juveniles, *Seriola quinqueradiata*, decreased schooling behaviour in turbidity due to impaired visual contact (Ohata et al. 2013). Shoaling facilitates information transfer and social learning between shoal mates about predator threats or food patches (Magurran and Higham 1988, Reader et al. 2003) as well as providing anti-predator behaviour, enhanced foraging efficiency or access to mates (Pitcher et al. 1982, Pitcher and Parrish 1993, Krause and Ruxton 2002). Reduced shoaling due to sensory disruption can result in decreased direct fitness due to reduced courtship interactions as I found in the second experiment described in this thesis (Chapter II). It can also lead to reduced food intake or higher predation risk and, hence, reduce individual fitness indirectly (Kelley et al. 2003). Juvenile coho salmon, *Oncorhynchus kisutch*, usually guarantee their food access by defending their territory, a behaviour which they gave up when they abandoned their social interactions in high turbidity (Berg 1983).

Inexperienced guppies showed strengthened associations to particular neighbours (Chapters I and II). The results of Chapter III, that inexperienced fish do not avoid risky areas in turbid water, however, suggests that information transmission is impaired and that strengthened associations to particular neighbours is not an adequate response to turbidity. I suggest that individuals potentially lingered with particular neighbours to avoid further separation from the group. This is further supported by the fact that inexperienced fish were less active in turbidity (Chapter I and II) which might help to keep fish together but it may also decline encounter rate among conspecifics (Fischer and Frommen 2013) and hence reduce access to social information. Further, it suggests that fish conduct less general sampling of their environment to keep updated (Webster and Laland 2011). In summary, the results suggest that reduced social interactions shown by inexperienced guppiesexperience turbidity as a constraint and may suffer fitness reduction and potentially population decline.

Increased social associations in experienced fish due to bigger group sizes and increased non-particular association at a cost of contacts to particular neighbours (Chapter II) suggests a stronger connectivity between all individuals within the population. It is likely that experienced guppies learned to better cope with turbidity and changed their social association pattern accordingly. Other species also show adjusted behavioural responses to human-induced environmental changes that facilitate better coping with the changed environment and counterbalance its effects on individual fitness. The nightingale, *Luscinia*

megarhynchos, sings at higher amplitudes in noisy environments compared to less affected territories and hence mitigates the impairments on their communication caused by masking noise (Brumm 2004). Great tits sing at a higher pitch to make their mating calls heard in noisy cities (Slabbekoorn and Peet 2003). Killer whales produce longer calls in the presence of noise from whale-watching boats in order to compensate for masked signals (Foote et al. 2004) and, under reduced short wavelength the western rainbow fish, *Melanotaenia australis*, changed its colour brightness and shoaled further apart to keep up communication through behavioural signalling (Kelley et al. 2012).

I argue that the changed structure to more connectivity (Chapter II) enabled experienced guppies to strengthen information transmission within the group under impaired visual conditions and with it to recover benefits of group living such as anti-predator behaviour (Chapter III). I did a focal follow observation, where one fish of each test group was assumed being an indicator for all fish in that treatment (Chapter III). Therefore, the response of the focal fish to the predator stimulus represents the response of all fish in the tank. There were ten fish in each test group. With the probability of 1:10, the focal fish itself detected the predator dummy first and hence avoided the potentially high predation risk area because of personal information. In all other cases, the focal fish must have been informed by shoal members about the location of the risky area to successfully avoid it. The information transmission process performed by experienced fish hence seems to be more effective than that of inexperienced fish when focal fish were not able to avoid the risky area. Individual fish may collect all possible social information mainly via non-particular associations to gather as much information as possible to counterbalance the impaired possibilities for personal information collection.

However, bigger group size combined with strongly reduced activity (Chapter III) may also influence predation risk in a different way. In conditions with poor visibility anti-predator behaviours of prey may be compromised since prey may not perceive the predator at a distance where anti-predator behaviour should already have been performed (Giske et al. 1994, Abrahams et al. 2007). Therefore, the only way for prey to control predation risk is to reduce encounter rates with the predator (Abrahams and Kattenfeld 1997). By exhibiting even greater reductions in activity in long-term turbidity (Chapter II), experienced fish can reduce encounter rates to an extent that it only depends on the activity of the potential predator. In addition, if prey density is high, the encounter rate is more frequent for the predator if prey is dispersed. Thus, prey should form bigger shoals to re-

duce encounter rate, particularly in turbid water, where long distance vision is impaired (Utne-Palm 2002, Turesson and Brönmark 2007).

Interestingly, the change in social association structure facilitated to maintain the number of courtship interactions (Chapter II) despite impairment of signalling and colouration of mates in experienced fish. Although the number of courtship interactions was maintained, mate choice may still be influenced due to switches to other senses under poor visual conditions as it is known in other species under human-induced environmental changes (Tuomainen and Candolin 2011). Guppies are known to use olfaction for food detection or to learn about predators (Hoare et al. 2004). In addition, females of the three-spined stickleback, *Gasterosteus aculeatus*, for example, relied more heavily on olfactory cues to choose males under turbid water conditions (Candolin et al. 2007). Such a switch to another sense may have wider implications for ecology and evolution. When female guppies got offered only olfactory cues for selecting males they were most attracted to different males than when offered visual cues (Shohet and Watt 2003). Similar results have been found in sticklebacks (Heuschele et al. 2009). These results suggest that switching to olfaction for mate choice, may lead to relaxation of visual selection pressure. Whether altered mate preference will lead to offspring better adjusted to new environmental conditions needs to be determined. The comparison of behavioural responses of differently experienced fish to turbidity and to ecological challenges under turbid conditions strengthen the conclusion of the first paragraph of the discussion that the responses of inexperienced fish are not an adjusted response but a constraint.

Behavioural responses to human-induced environmental changes can take place at different time scales. The shortest time scale occurs with the initial response to the first encounter with environmental change. At intermediate time scales, a response may be learned over time resulting in behaviour that facilitates coping with the environmental change. And finally, at the longest time scale changes in behavioural patterns may be fixed through genetic changes (Tuomainen and Candolin 2011, Sih 2013). In the present thesis, I addressed the first two levels of response. Initial behavioural responses to human-induced environmental change can be well adapted to new environments or maladaptive. The decrease in social associations exhibited by inexperienced fish was partly caused by fish being alone longer (Chapter I and II) and hence caused dispersal of the fish. Dispersal is a typical initial behavioural response to environmental change (Tuomainen and Candolin 2011). Considering the impact of reduced overall associations for direct and indirect fitness that

may occur, the initial plastic response does not seem to be optimal for the new turbid environment. Nevertheless, in guppies' natural habitats, turbidity occurs regularly but not continuously, which might enable the fish to learn to cope with turbid conditions. Yet, although initial behavioural responses were not perfectly adjusted to environmental changes (Chapters I and II), this may give the fish additional time to learn how to better cope with turbidity which was be observed in experiments in Chapter II and III. Responses at later stages of environmental change as turbidity-experienced fish showed can be due to the social transmission of gradually changing behavioural reactions and to genetic alterations of the behavioural reaction norms (Tuomainen and Candolin 2011). Whether the results represent a genetically based behaviour or are learned altered behaviours cannot be disentangled with my results, as I did not explicitly test for it. However, as experienced and inexperienced fish showed similar behaviour in clear water (Chapter II) this might indicate the absence of genetic differentiation among the populations. In young African cichlid, *Pseudocrenilabrus multicolor victoriae*, it has been shown that rearing in turbidity was enough to change total behaviour and no influence of the origin was detected (Gray et al. 2012). However, since behavioural responses depend on the information that individuals acquire regarding their surroundings, changes in the sensory environment can have profound effects on the behaviour of individuals and consequently on their fitness.

Conclusion and perspectives

This study was conducted on a model organism that has been used for research in the field of evolution, ecology and behaviour for many decades. In this study I found that human quarrying activities resulting in high turbidity levels strongly influence the behaviour in social structure and the mechanism for coping with ecological challenges in guppy populations. This may influence the viability of the individuals and hence may affect the population distribution.

While previously described results suggest that changed social structure supports coping with predation risky situations, the mechanism of this strengthened connectivity is still not understood. However, video clips of the food and predator stimulus experiment described in Chapter III have been gathered. It would be interesting to investigate the social cohesion within the groups in experienced and inexperienced fish during food and preda-

tor stimulus treatment. Tracking software could be used to measure the distances between individuals in experienced and inexperienced fish, which would give deeper insight into the information transmission process.

The ecological tasks of food finding and predator avoidance could be used for further investigations into information transmission, to determine whether exclusively visual stimuli are used or if olfactory stimuli are also involved, as is the case in mate selection by the poeciliid fish *Xiphophorus pygmaeus*, which is known to use vision and olfaction (Coleman 2011) and in the fountain darter, *Etheostoma fonticola*, where it has been shown that only the chemical sense is not enough to show anti-predator behaviour (Becker and Gabor 2012). To use experimental designs with predator and food dummies with and without olfactory cues combined could produce deeper insights.

Given that many, if not most, streams in Trinidad are increasingly affected by quarries and deforestation (Agard et al. 2004), changes in shoaling behaviour should be monitored. However, increased turbidity caused by quarrying does not appear on its own but is often associated with rainfall in streams and rivers and therefore often accompanied by periodically higher water levels and stronger currents (Maya et al. 2012). Strong currents can potentially displace fish and flash floods leave the river bottom and all objects in the stream covered in a thick coating of mud. Little is known how these flash-flood events affect the Trinidadian guppy and what influence the increasing sedimentation has on the primary productivity of streams. Deforestation can increase the effect of stronger currents and can also lead to higher nutrient concentrations and higher stream temperatures (Likens et al. 1970). Anecdotal observations at the Turure River over the last 10 years indicate that deforestation has increased primary productivity of the streams due to increased sunlight (reaching the river beds) leading to higher population densities of guppies and larger individuals. Systematic scientific studies on human-induced environmental changes of such trends in Trinidad are urgently needed because they are likely to affect the ecology and evolution of guppy populations which have been a focus point of scientific attention for more than 50 years.

Change of perspectives

I would like to take the reader to a short trip and shift the focus to a different, but related topic. In my thesis, I studied the social response of fish to turbidity. I found that turbidity-inexperienced fish decreased the number of associations and performed poorly during a

predator threat. On the other hand, fish with previous experience with turbidity formed stronger connectivity within their groups and coped better with a predator threat. From these results, I concluded that stronger connectivity led to a better performance when fish faced an ecological challenge. Apparently, the usual behaviour repertoire and problem solving mechanisms of the inexperienced fish were not appropriate to adjust to a change in environmental condition, while experienced fish adjusted by strengthening connectivity. Although from a different discipline, the response of inexperienced fish is in accordance with the crisis definition of the social psychologist Gerald Caplan (1964). He states that a crisis arises when the usual behaviour repertoire and problem solving mechanisms are triggered by an event, but that these normally preventative measures are not sufficient to cope with the internal and/or external factors of said event. The population is then forced to adopt a novel approach. As an environmental change, turbidity plays the role of just such an external factor.

In times of global and climate change, the world is facing rapid and drastic environmental and social changes in the near future. If we are not to be simply swept along, but rather to direct these changes and develop new strategies within a Great Transformation (WBGU 2011), we will need to use all available sources of inspiration. Science has led the world in adopting an inter-disciplinary approach to problem solving in research and development. Animal models have long been in use for predicting human responses in the field of medicine. Perhaps we can use our insights on the response of animals to drastic environmental changes to develop hypotheses for human societies.

I am aware that the human behaviour is overlaid with various systems such as the economic-technological system, or by political and cultural values. Additionally, mechanisms of animal social systems are not directly transferable. However, some principles in human and animal social systems are similar. For example, individuals have their own personal horizon of experience and limited information access. Based on their specific informational background, individuals evaluate situations as benefitting or impairing, resulting in a respective response (Weber 2007). Hence, experienced guppies and their response to environmental change implies comparing people who have previously experienced environmental catastrophes and developed closer ties to other members of the society to those that do not and see who can better cope with catastrophes. If this turns out to be the case, it may imply that societies, which are better connected, are also better prepared for environmental catastrophes than less connected ones, whether or not they have previously

experienced a catastrophe. With this in mind, we could raise the question of how people could better connect, prior to or without ever having experienced a catastrophe, to be prepared.

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Chapter I Turbidity affects social dynamics in Trinidadian guppies

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Karoline K. Borner, Stefan Krause, Thomas Mehner, Silva Uusi-Heikkilä, Indar W. Ramnarine and Jens Krause

Turbidity affects social dynamics in Trinidadian guppies

Karoline K. Borner[1,5], Stefan Krause[2], Thomas Mehner[1], Silva Uusi-Heikkilä[3], Indar W. Ramnarine[4] and Jens Krause[1,5]

[1]Leibniz-Institute of Freshwater Ecology and Inland Fisheries Müggelseedamm 310, 12587 Berlin, Germany

[2]Department of Electrical Engineering and Computer Science, Lübeck University of Applied Sciences, 23562 Lübeck, Germany

[3]Department of Biology, University of Turku, 20014 University of Turku, Finland

[4]Department of Life Sciences, The University of the West Indies, St Augustine, Trinidad & Tobago

[5]Humboldt Universität zu Berlin, Faculty of Life Sciences, Invalidenstraße 42, 10115 Berlin, Germany

Abstract

Turbidity, caused by suspended particles in the water column, induces light scattering and shifts in the wavelengths of light. These changes may impair the ability of fish to use physical cues and hence may modify social interactions. We experimentally investigated the social interactions of guppies, *Poecilia reticulata*, in clear and turbid water. Fish were significantly less active, formed smaller shoals and were found to be more often alone in turbid than in clear water. A Markov chain analysis revealed significant differences in the social dynamics when comparing clear and turbid water conditions. The probability of leaving a particular nearest neighbour and the probability of choosing some neighbour after swimming around alone differed between the treatments. Our results indicate that turbidity has a number of different effects on the social interactions of the guppy, and we discuss their potential costs and benefits and wider implications.

Keywords Social dynamics, shoaling behaviour, *Poecilia reticulata*, group-living, turbidity

Introduction

Turbidity in the water body has become a worldwide phenomenon and is steadily increasing. It is caused by suspended particles that generate light scattering. This is mainly a result of anthropogenic influences (Scheffer et al 2001). Intense human land use like deforestation, mining and agriculture lead soils releasing clay and silt particles and increased amounts of fertilizers, which are washed into rivers and lakes. While fertilizers raise productivity of aquatic ecosystems, often reflected in substantially increased algae growth (Carpenter et al. 2001; Conley et al. 2009), soils, clay and silt may be suspended in the water column and hence may cloud the water (Tockner et al. 2010). Quarrying is one of the human activities that affects land and has lasting effects on the environment (Maya et al. 2012). With increasing demand for construction material in the centres of dense population and development all over the world, the consequence of quarrying moves into focus. Closely linked but not mandatory to the activity of quarrying is deforestation. Both can lead to higher nutrient concentrations, pH-values, temperatures and stronger currents in streams (Likens et al. 1970; Galas 2003). With flash floods, clay from the quarries and soil can be washed into the rivers leaving a thick coating of mud and high turbidity levels

(Tockner et al. 2010; Maya et al. 2012) which might influence the physiology of organisms (Ardjosoedirо and Ramnarine 2002). In addition, turbidity scatters and reduces the amount of light and may lead to a change of the light spectrum, in the water column (Utne-Palm 2002). Many fish species rely on vision as their main source of information, for example with respect to foraging and anti-predator behaviour (Guthrie and Muntz 1993). Therefore, changes in the natural environment that modify the optical properties of water or the visibility per se may have important consequences for the biology of fishes (Endler 1992; Utne-Palm 2002; Chapman et al 2009; Chapman et al 2010; Sluijs et al 2010).

In fish, visual communication plays a role in kin recognition and familiarity (Griffiths and Magurran 1999; Hain and Neff 2007), identification of predators and social learning (Laland and Williams 1997; Day et al. 2001) as well as foraging and shoaling behaviour (Hoare et al. 2000). In highly turbid water, visibility may be reduced and a decrease of information exchange may occur. Fish may be able to counteract such constraint by increasing their activity. Such a response has been found in juvenile cod (*Gadus morhua*) where fish under turbid conditions kept their foraging rate constant by increasing their activity levels (Meager and Batty 2007). In the presence of predator cues, pike larvae (*Esox lucius*) hid within the vegetation less and exploited the open water more when under turbid conditions than when under clear water conditions (Lehtiniemi et al. 2005). However, African cichlid showed a marginally significant decrease in activity in turbid water when fish were reared in turbidity (Gray et al 2012).

To investigate the influence of turbidity on social behaviour, we selected the Trinidadian guppy, *Poecilia reticulata,* which has become a classic study system in behavioural ecology and evolutionary biology over the last 50 years (Magurran 2005). The guppy is subject to high levels of turbidity in large parts of the Northern Mountain Range of Trinidad as a result of quarrying activities. After rainfall, runoff from quarries enters the rivers causing dramatic short-term increases in turbidity (KKB and RJGC pers. obs.). In our study, we examined changes of activity and social behaviours in guppies exposed to turbidity. To investigate the changes of social structure, we carried out generalized linear mixed model. The social dynamics of the fission-fusion process was analysed with the Markov chain approach. We hypothesised that increased turbidity would have an effect on the activity of the fish as well as on the association behaviour with conspecifics and social dynamics. We predicted that the activity of the fish would differ under clear and turbid water conditions. Further, we predicted that group size would decrease and the frequency of fish being

alone would increase in turbid water because encounter probabilities between individuals should be reduced in turbidity.

Methods

Study species

Guppies caught from the Northern Mountain Range of Trinidad, Arima River, were bred for about 20 generations in the laboratory. They were fed twice a day with TetraMin dry food. Water had a constant temperature of 24° C, and diel illumination rhythm was at 12/12 h.

Experimental setup

One hundred fish (50 males, 2.2-2.8 cm; 50 females, 2.8-3.8 cm) were individually marked with fluorescent elastomer which are known to have no impact on fish behaviour (Croft and Krause 2004). Fish were divided into 10 groups of 10 fish (five males and five females) each and were kept for 12 days in 40 x 30 cm tanks to become familiar with each other (Griffiths and Magurran 1997). Subsequently, the groups were transferred to test tanks (100 x 100 x 20 cm with a grid of 10 x 10 cm squares placed below the tank to facilitate the recording of two-dimensional positions of individual fish) and left overnight for acclimation either in clear or in turbid water. The next day, each group of 10 fish was observed for 2 x 30 min. After observation, groups were transferred to test tanks with the opposite water quality treatment and left overnight for acclimation. Observations were conducted the next day. Half of the groups experienced turbidity first, and the other half experienced clear water first to control for treatment order. To facilitate individual recognition of fish during experiments from above even under turbid conditions, water depth was limited to 2 cm. Guppies often enter very shallow waters in the field (KKB and JK pers. obs.); therefore, such shallow water does not provide unusual conditions for them. Furthermore, pilot experiments showed that the fission-fusion dynamics of fish in tanks was consistent with that shown in the wild (see Wilson and Krause et al. 2014). Turbidity was created using 5 g of Kaolin (Amberger Kaolinwerke, Germany) to achieve a level of about 1000 nephelometric turbidity unit (NTU). We used Kaolin to turbid the water as it consists of little clay particles with a big surface that keeps the particles suspended for a long time (Meager et al. 2005). Turbidity levels dropped to 700 NTU during experiments but the optical range of the fish was consistently lower than 5 cm (pre-test showed that fish reacted towards other

fish only within a 5-cm range). For comparison, turbidity levels in the clear water treatment were about 5-10 NTU. Similar NTU ranges, namely about 900 -1000 NTU for turbid and about 7 NTU for clear water, were measured in natural guppy habitats at the Turure River, Trinidad. Turbidity was measured with Horiba Water quality checker U-53, Kyoto, Japan. To avoid sedimentation of Kaolin during the experiments, we gently stirred up the bottom of the tank in the evening, in the morning and before each observation period. We did so in both treatments to control for potential disturbance of fish.

Data collection

Each fish (in a group of ten) was individually tracked by direct observation for 2 x 3 min per treatment. The position of the focal fish on the grid and the identities of its neighbours were recorded every 10 s giving 2 x 18 data points per focal fish. We defined a focal fish's neighbours as follows: first, all fish which were within four body lengths of the focal fish (which is a commonly used criterion for social associations, Krause & Ruxton 2002) and second, all fish which were within four body lengths of the above mentioned neighbours (if present). The 10-s duration and the observation time of 3 min were chosen because previous work indicated that this approach captures their social dynamics (Wilson and Krause et al. 2014).

The following response variables were calculated:

1. Mean shoal size. We calculated the arithmetic mean of shoal size for each focal fish out of the number of its neighbours from 2 x 18 data points.
2. Mean time alone. Out of the 2 x 18 data points for each focal fish, we recorded how many times a fish was observed alone, i.e. no other fish was located within four body lengths of the focal fish. From these counts, we calculated the arithmetic mean.
3. Mean activity. If a fish changed its grid position (as measured by the squares under the tank) from one 10-s recording to the next, we defined this as a square crossing (regardless of the number of squares moved). We calculated the mean activity as the mean number of square crossings of each fish.

Statistical analysis

From the original 10 groups, one group had to be excluded because of incomplete data. Furthermore, two single male fish from separate groups had missing data or died before

the first observation. Therefore, the subsequent analysis was carried out with 88 fish in nine groups. To evaluate whether social behaviour of guppies differed between the treatments and fish sexes, we calculated generalized linear mixed models (GLMM). Models were constructed with treatment and sex as fixed factors, and individual fish nested within groups as a random factor. All response variables were approximated as count data; therefore, we applied the Poisson family distribution with its canonical link function (Kachman 2000). Correlations between activity and solitary fish and activity and mean shoal size were calculated by Pearson's *r*. All statistical calculations were performed by the function glmmPQL from MASS package (Venables and Ripley 2002) in R version 2.14.1 (R Core Team 2011) and Sigma Plot 12.0.

Markov chains

In order to better understand the underlying dynamics of the patterns of social behaviour and to investigate differences between the two treatments, we used a fission-fusion model based on Markov chains. Following Wilson and Krause et al. (2014), we constructed two variants (with different levels of detail) that describe the social behaviour common to all focal individuals as sequences of 'behavioural states'. In the simple model (Figure 1a), a focal fish can either be social (with a conspecific) denoted by *i* or alone (no conspecific within four body lengths) denoted by *x*. By regarding *i* and *x* as states of a first-order Markov chain, the transition probabilities between these states can be estimated from the data points in our observations (see supplementary material and Wilson and Krause et al. 2014 for more details). Our simple model then predicts the distributions of the lengths of phases of social contact and of being alone. In the more detailed model (Figure 1b), the identities of the neighbours of the focal fish are also taken into account, i.e. the state *i* of the simple model is split up into *k* states i_1, ..., i_k, where *k* is the number of potential neighbours and the state i_j indicates that the focal fish is currently associated with individual *j*. The more detailed model has *k*+1 states, one for each potential neighbour plus the state *x*. In addition to the simple model, the more detailed model predicts the lengths of contact phases with the same neighbour.

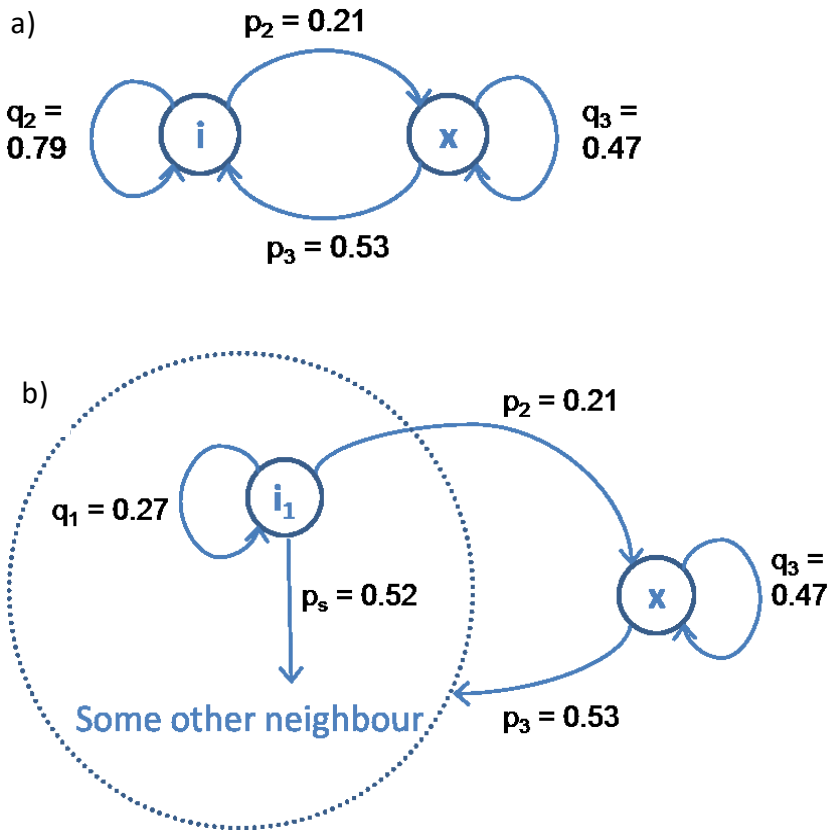

Figure 1 Markov chain models of social behaviour in the guppy. All probabilities are estimates for the clear water treatment. a) The simple model. A fish can either be social (with a conspecific) denoted by i or alone (no conspecific within 4 body lengths) denoted by x. p_2 and p_3 indicate the respective probabilities for changing states and $q_2 = 1 - p_2$ and $q_3 = 1 - p_3$ those for retaining current states. b) The more detailed model. In this model, state i (dotted circle) is split up into as many states as there are potential neighbours, and the focal fish is in state i_g, if individual g is its nearest neighbour. In our model the dynamics do not depend on the neighbour's identity and is the same for each state i_g. Therefore, for the sake of clarity the figure only shows the state i_1. A focal fish stays with its current nearest neighbour with probability q_1. When the contact with this neighbour ends it decides to be alone with probability p_2 or switches to a different neighbour with probability p_s. If there are k potential neighbours other than i_1, the probability of choosing a particular one is p_s/k.

In a first-order Markov chain, the next state only depends on the current state. In our case, this means, for example, that the decision of a focal fish to leave its current neighbour and to swim around alone (only) depends on its current state. Wilson and Krause et al. (2014) showed that this is a valid assumption for female guppies observed in the wild.

In the supplementary material (Figure 4, Figure 5), it is demonstrated that the simple and the more detailed Markov models capture the dynamics of the state changes in our observations and that the observed lengths of social contact, of contact with a particular nearest neighbour and of being alone are well approximated by the model predictions. The model probabilities were estimated as simple proportions (see supplementary material for more details). This allowed us to investigate differences between the treatments

regarding the lengths of social contact phases, of contact phases with the same neighbour and of being alone, where the length was measured as multiples of time intervals of 10 s. We used the two-sample test for equality of proportions with continuity correction in R for our tests.

Results

The mean shoal size was significantly greater in clear than in turbid water (Table 1; Figure 2a), and fish in turbid water were significantly more often alone than fish in clear water (Table 1; Figure 2b). Furthermore, fish in clear water were significantly more active than fish in turbid water (Table 1; Figure 3). Males and females differed significantly in mean activity across both treatments with males showing higher levels of activity (Table 1). Therefore, we investigated the potential influence of activity on social behaviour. We found no significant correlation between activity and mean shoal size (Pearson correlation, r=-0.364, p=0.335) nor between activity and mean time alone in turbid water (r=0.293, p=0.445). Furthermore, no significant correlation was found between activity and mean shoal size (r=-0.488, p=0.182), nor between activity and time spent alone in clear water (r=0.513, p=0.158).

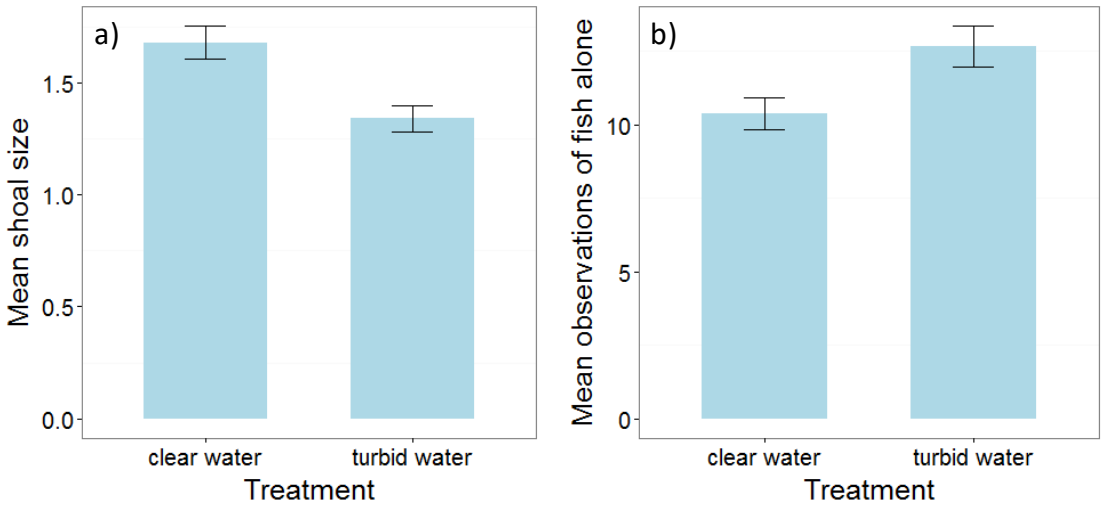

Figure 2 Shoaling behaviour of the fish in turbid and clear water. a) The mean shoal size and b) the mean time fish were observed alone. Bars show mean values and standard error calculated for n=176 fish.

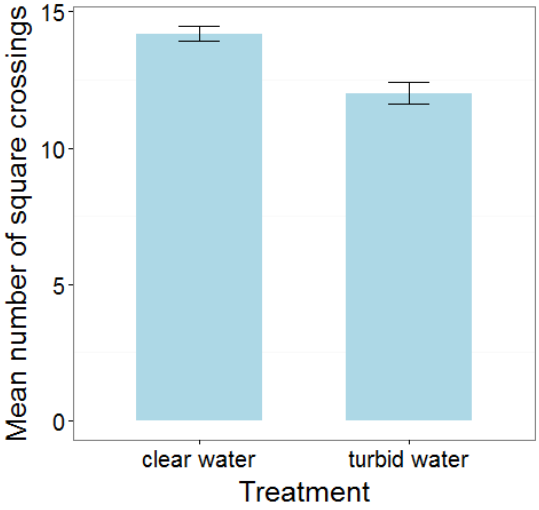

Figure 3 Comparison of activity (mean number of squares fish transited out of 2x17 possible) between turbid and clear water treatments. Bars show mean values and standard error for n=176 fish.

Table 1 Results of GLMM with treatment, sex and their interaction as fixed factors and fish nested within groups as random factor.

Response variable	Fixed factor	Mean	Std. Error	DF	t-value	p-value
Mean shoal size	treatment	-0.1941	0.0699	87	-2.7760	0.0067
	sex	0.0016	0.0668	79	0.0233	0.9815
	treatment*sex	-0.0368	0.0999	87	-0.3681	0.7137
Mean time alone	treatment	0.2065	0.0930	87	2.2199	0.029
	sex	0.1386	0.1065	79	1.3011	0.1970
	treatment*sex	-0.0290	0.1283	87	-0.2256	0.8220
Mean activity	treatment	-0.2087	0.0531	87	-3.9345	0.0002
	sex	0.1167	0.0503	79	2.3201	0.0229
	treatment*sex	0.0961	0.0724	87	1.3271	0.1880

The Markov model probabilities were computed across all groups (Table 2) for each treatment (clear and turbid water). The probability p_s of switching the nearest neighbour (0.52 in clear water and 0.43 in turbid water) and the probability p_3 of choosing some neighbour after swimming around alone (0.53 in clear water and 0.38 in turbid water) differed between the treatments. Both probabilities were significantly greater in clear water than in turbid water (two-sample test for equality of proportions with continuity correction, both p values < 0.001). The probability of ending social time (i.e., leaving any neighbour) did not

differ significantly between the treatments (Table 2). This means that both the contact phases with a particular nearest neighbour and the phases of being alone were longer in turbid water than in clear water, while the lengths of general social contact did not differ.

The reciprocal values of the model probabilities specify the mean number of data points over which a focal fish will retain a state. Multiplying this number by 10 s (the time between two data points) yields the mean time spent in each state. The mean length of social contact in clear water was about 47 s and in turbid water about 50 s. Social contacts included 3.4 different nearest neighbours in clear water and 3.2 in turbid water on average. The mean length of contact with a particular nearest neighbour was 14 s in clear water and 16 s in turbid water.

Table 2 Estimated model probabilities p_1, p_s, p_2, *and* p_3 *and 95% confidence intervals for both treatments.* p_1 *is the probability of leaving the current neighbour,* p_s *of switching to a different neighbour,* p_2 *of leaving any neighbour (i.e. switching from state* i *to state* x*), and* p_3 *of choosing some neighbour after having been alone (i.e. switching from state* x *to state* i*). Note that* $p_s = p_1 - p_2$*. The corresponding probabilities* q_1, q_2, *and* q_3 *of retaining the respective states are not contained in the table because* $q_j = 1 - p_j$ *for all* j*. The p-values resulted from the 2-sample test for equality of proportions with continuity correction.*

Treatment	**Clear water**		**Turbid water**		
	Estimate	*95% CI*	*Estimate*	*95% CI*	*p-value*
P_1	0.73	0.71 - 0.75	0.63	0.61 - 0.65	< 0.001
p_s	0.52	0.50 - 0.54	0.43	0.41 - 0.45	< 0.001
P_2	0.21	0.20 - 0.23	0.20	0.18 - 0.22	0.25
P_3	0.53	0.50 - 0.57	0.38	0.35 - 0.41	< 0.001

Discussion

Our results showed that turbidity leads to a strong behavioural response, which has potentially important implications for information transfer and predator recognition. Furthermore, females and males differed in activity levels.

Altered social behaviour in turbid water may be a result of a sensory constraint in that fish are less able to detect conspecifics visually. Therefore, individuals cling longer to a particular neighbour or remain longer alone after leaving their neighbour, leading to smaller group sizes. Smaller group sizes and less frequent switching of nearest neighbours

suggest that guppies under turbid conditions might have less access to public information and fewer opportunities for social learning about food locations (Laland and Williams 1998; Bates and Chappell 2002) and identities of predators (Magurran and Higham 1988). Lower activity rates suggest that fish conduct less general sampling to keep up to date with their surroundings (Webster and Laland 2011).

Smaller group size and longer time alone could also be interpreted as an adaptive response to changed environmental conditions. Several studies suggest that turbidity might reduce the perceived risk of predation in fish from visually hunting predators (Gregory and Northcote 1993; Maes et al 1998). In studies of Pacific salmon (*Oncorhynchus tshawytscha*) it was shown that fish perceived less predation risk in turbidity and foraged more often in open water in turbidity than in clear water (Gregory 1993; Gregory and Levings 1998). Furthermore, Fischer and Frommen (2013) showed that in turbidity the three-spined stickleback (*Gasterosteus aculeatus*) lost its preference for bigger shoals. Smaller group size and more time alone in turbidity could be therefore an adjustment to reduced perceived predation risk (Abrahams and Kattenfeld 1997). In addition, competition for food is reduced in smaller groups (Hoare et al 2004).

With the Markov chain approach, we were able to shed more light on the dynamics behind the observation that fish are more often alone in turbid water than in clear water. Obviously, the fish did not increase the number of phases of being alone but rather their lengths, supporting our first hypothesis that turbidity might be experienced as a constraint.

It has been proposed that under turbid conditions predator-prey interactions are primarily characterized by the encounter rate of predator and prey (Abrahams and Kattenfeld 1997). In clear water, prey can watch for predators and use anti-predator behaviour for protection whereas the only chance under high turbidity and short recognition distances is to avoid encounter. Therefore, decreased activity interpreted as an active response to turbidity would allow fish to reduce actively the encounter probability with predators. A supporting result was found in sticklebacks where individuals switched less often between shoals in turbid water (Fischer and Frommen 2013). Generally, males were found to be more active than females. Since females are receptive approximately every four weeks, a reasonable interpretation might be that males are more active to find new mates (Magurran 2005).

Fish showed a strong behavioural response to turbidity in our study. Such behavioural flexibility might be used to minimize negative effects in order to cope with environmental change (Wright et al 2010; Sih et al 2011; Tuomainen and Candolin 2011). In fact, a complex behavioural and cognitive repertoire is known to influence the probability of colonization (Huizinga et al 2009; Bisazza 2011) and the guppy is probably the world's most widely distributed tropical fish (Magurran 2005). However, regular occurrence of turbidity implies regular disturbance of the social dynamics of fish and they might learn to better cope with it and develop different strategies (Sih 2013). For example, guppy males are known to be selected for colourful pigments by females (Houde 1997; Magurran 2005). Under regular occurrence of turbidity, females may switch to another sense for mate choice, for example olfaction, reducing visual selection pressure as it has been shown in sticklebacks (Heuschele et al 2009). Cichlid flocks in the great lakes of Africa have also been strongly affected by turbidity within the last few decades. Cichlids showed a strong radiation where species were sexually isolated by visual mate choice. With increasing and enduring turbidity caused by algae blooms due to eutrophication, species radiation collapsed. Cichlid females were not able to differentiate between different colour morphs of males anymore (Seehausen et al 1997). Here, females, apparently, were not able to switch to another sense to maintain speciation.

However, not only the visual sense of fishes are impacted by anthropogenic influences. Road traffic, for example, masked females perception of male signals in the grey tree frog, *Hyla chrysoscelis*, (Bee and Swanson 2007) or provoked altered singing frequencies in the grey shrike-thrush, *Colluricincla harmonica* (Parris and Schneider 2009). In bottlenose dolphins, *Tursiops Sp.*, communication range was found to be reduced by vessel noise (Jensen et al 2009).

With our study, it is not possible to disentangle whether we observed an active behavioural change to turbidity or whether it acted as a constraint. However, Trinidad is increasingly affected by quarries and deforestation (Agard et al. 2004) and changes in social behaviour of affected fish should be closely monitored. Even more when we take into account that turbidity induced by quarry runoffs has many other effects, such as periodically higher water levels and stronger currents associated with rainfall. Further systematic studies are urgently needed to understand how regularly occurring environmental changes affect the behaviour, ecology and evolution of guppy populations, which have been a focus point of scientific attention for more than 70 years (Houde 1997). This will also require

novel methods to assess changes in behaviour as a result of disturbance. In our study, we used a first-order Markov chain (MC) as an individual-based model to approximate the social dynamics of guppies. A MC represents the simplest possible individual-based model and provided a good fit to our data. There is a twofold benefit of modelling fish social behaviour in this way. Firstly, the MC captured the dynamics of the social behaviour, unlike most network approaches which are based on accumulating observations on such a coarse temporal scale that the dynamics are lost (Sundaresan et al. 2007; Henzi et al. 2009). Second, we can circumvent some of the problems associated with conventional social network comparisons because MCs avoid the confounding influence of differences in network density (Croft et al. 2008). The fact that the MC modelling approach worked for guppy social behaviour in both the lab (this study) and the field (Wilson and Krause et al. 2014) makes it a promising candidate for future studies that extend to other contexts (than shoaling behaviour) and to other species.

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Turbidity affects social dynamics in Trinidadian guppies

Modelling approach and parameter estimation

Our observations consist of sequences of 'behavioural states'. At each time point a focal fish can either be social (with a conspecific) denoted by i or alone (no conspecific within four body lengths) denoted by x. Our task is to find a model that captures the dynamics of these sequences. The simplest possible approach would be to use a single parameter p that specifies the probability of the next state being i. This parameter can be estimated by computing the relative frequency of the state i. However, this very simple approach is based on the unlikely assumption that the next state is independent of the preceding states. Wilson et al. (2014) have shown that this assumption cannot be made for guppies in the wild (see also the below section on the BIC analysis). Following their approach, we introduced conditional probabilities into our model such that each state depends on the immediately preceding state. More specifically, we used the two probabilities

$$p_2 = \text{Prob}(\text{state}_{n+1} = x \mid \text{state}_n = i), \text{ and}$$
$$p_3 = \text{Prob}(\text{state}_{n+1} = i \mid \text{state}_n = x).$$

Here, p_2 (p_3) denotes the probability that the focal fish switches to state x (i) when its current state is i (x). Both probabilities can be estimated based on relative frequencies (Fink 2008). Figure 1a in the main text shows a graphical representation of the resulting model. Its probabilities define the transition probabilities of a (first-order) Markov chain with states i and x without having to introduce any further assumptions or parameters. This allows predictions that will be used in the following sections to analyse the goodness of fit of our model.

If a focal individual has more than one potential neighbour, it can switch between them while staying social. The more detailed model (Figure 1b in the main text) extends the simple model by additionally describing the internal dynamics of the state i. This means, both models are exactly the same, if the switching behaviour is ignored. The more detailed

model adds one parameter to the simple model, the probability p_1 of leaving the current neighbour. Like p_2 and p_3, p_1 is a conditional probability,

$$p_1 = \text{Prob}(\text{state}_{n+1} \neq i_k \mid \text{state}_n = i_k).$$

It can be estimated in the same way as p_2 and p_3, i.e. based on relative frequencies. The probability p_s for switching the neighbours while staying social can then be computed as follows:

$$p_s = \text{Prob}(\text{state}_{n+1} = i_l \mid \text{state}_n = i_k), \text{ where } i_l \neq i_k \quad = \quad p_1 - p_2.$$

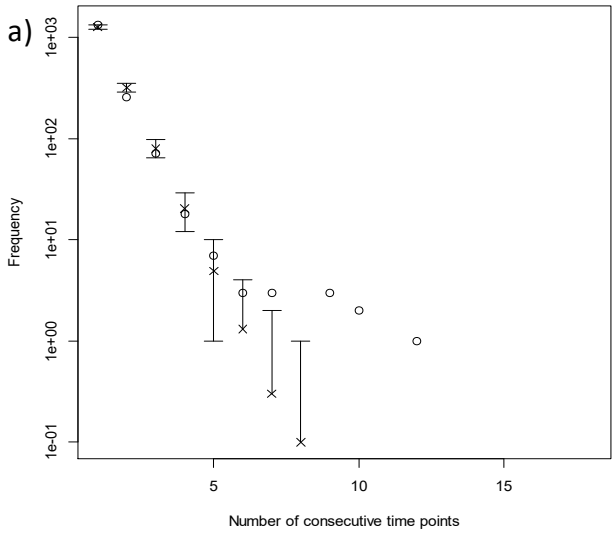
Goodness of fit of the Markov chain model

The time spent in a state of a Markov chain follows a geometric distribution. In our study system this means, the frequencies of phase lengths of being social or alone should decrease exponentially with increasing phase length. To compare the model predictions with the observed data we simulated observations of the model's behaviour where we took into account the 3 min observation time per focal individual. This is necessary because incompletely observed phases (that started or ended outside the observation period) will lead to higher numbers of short phases than theoretically expected. We repeated the simulation 10^4 times and computed the mean frequencies and the 2.5% and 97.5% percentiles for each phase length. The simulation was based on the estimated probabilities and did not take into account their confidence intervals. Therefore, the predicted percentile ranges are conservative. Our results show that the observed data are well approximated by the model predictions (Figure 4). A few deviations concerning long contacts exist but this has to be seen in the context of the very low frequency of such unexplained contact lengths and the relatively conservative model predictions.

Another prediction of a Markov chain model regards the so-called mixing time (see, for example, Levin et al. 2008). For our study system this means that after a small number of time steps the probability of being in state *i* or state *x* should be (almost) independent of the initial state. In other words, regardless of whether an individual was in state *i* or state *x* at the beginning of a sequence, after a small number of time steps it should be in state *i* with a certain probability π(i) and in state *x* with probability 1 - π(i). We used this prediction for a test of the goodness of fit in the following way. We performed 10^5 simulations of our model and determined the frequency distribution of the states *i* and *x* after the *n*-th time step for each of the initial states *i* and *x* separately. Theoretically, these distributions

should be approximately equal after the 5th time step. We measured their difference using the total variation distance (which is the maximum difference between the probabilities assigned to a single event by the two distributions, Levin et al. 2008). To make the numbers comparable with our observed numbers we started each simulation such that the initial states were identical to those in our observations. Figure 5 shows the goodness of fit for our simple model. In accordance with theory independence of the initial state is reached after about 5 steps and most of the observations are well within the boundaries of the 95% percentiles (as predicted by the Markov model). To demonstrate that this is not self-evident, the figure additionally shows the 'goodness of fit' for a fictitious scenario, where the 'true' behaviour did not follow a Markov chain but where the lengths of phases were uniformly distributed in the range 4-6 time points for being social and 1-2 time points for being alone. In this case, deviations from the model predictions are clearly visible with many values exceeding the boundaries of the 95% percentiles (as predicted by the Markov model) (Figure 5).

We performed the mixing time analysis only for the simple model with two states. For the more detailed model the number of states depends on the number of potential neighbours and more data is required to be able to detect deviations from the predicted mixing time. Instead, we tested whether the mean lengths of contact with a particular neighbour differed between the individuals. The more detailed model assumes that there are no such differences. To test this we applied a randomization test where the identities of the focal individuals and their neighbours were randomized. As a test statistic we used the variance of the mean length of contact across all pairs of individuals. This test statistic yields large values, if the mean lengths differ between the individuals. The results showed that this was not the case in our observations (10^4 randomisations, $p = 0.64$ in clear water and $p = 0.73$ in turbid water).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5
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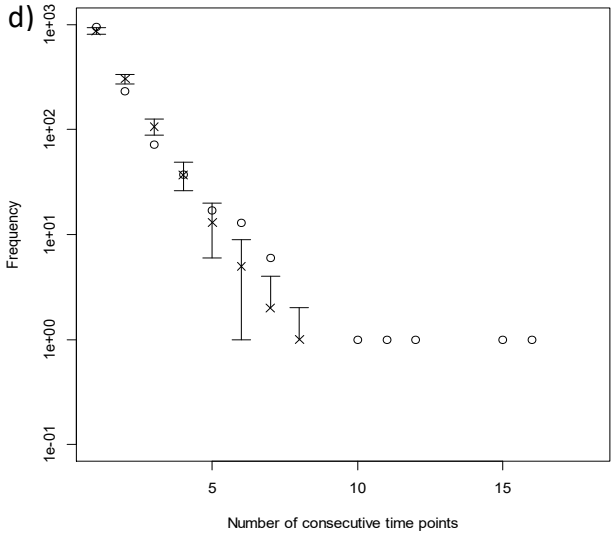
Number of consecutive time points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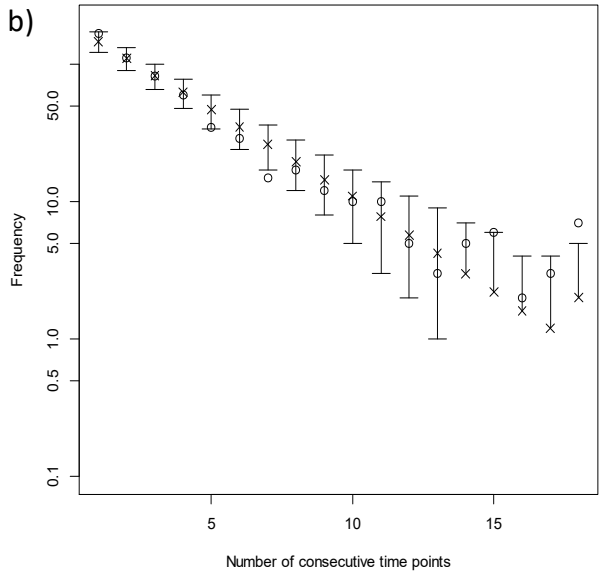
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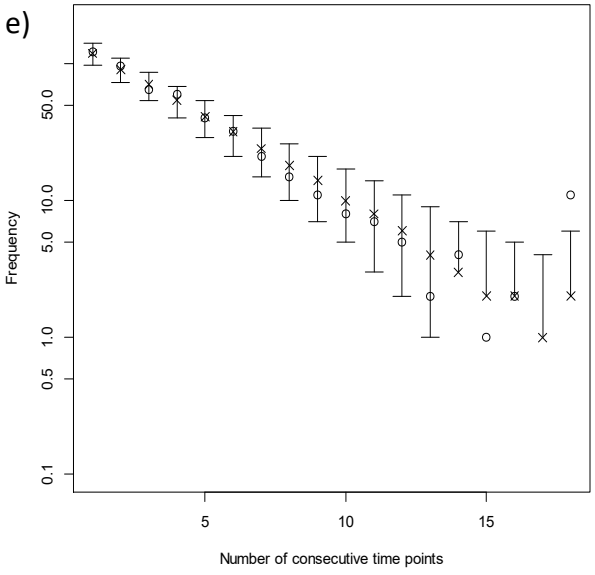
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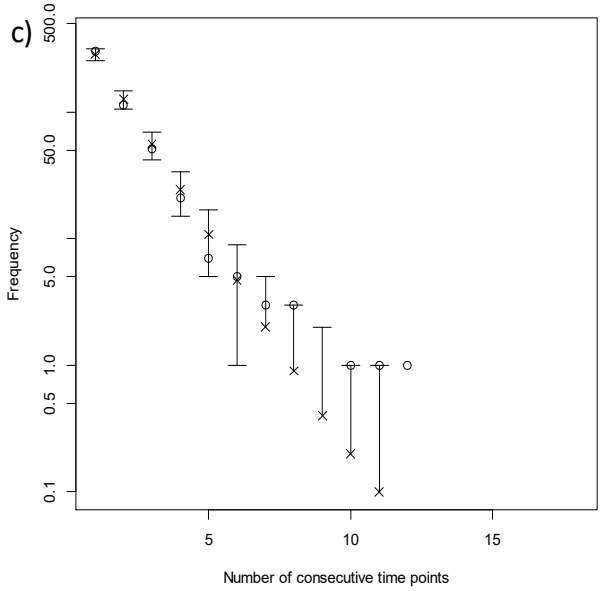
c)
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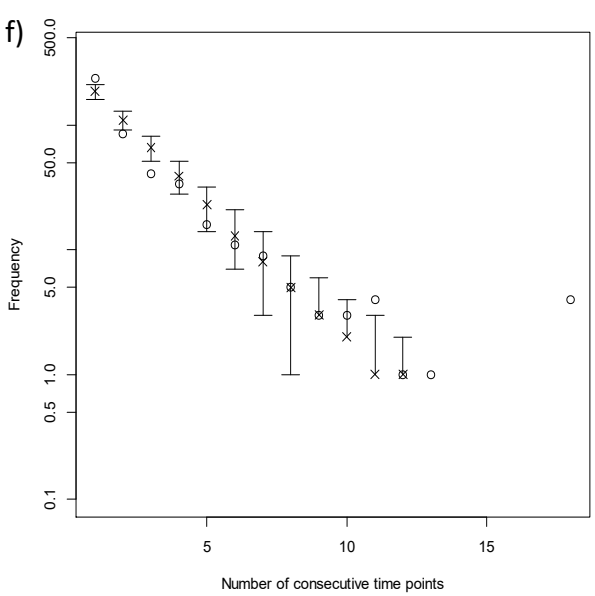
Number of consecutive time points


f)
Frequency
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50.0
500.0
5
10
15
Number of consecutive time points

Figure 4 Frequency distributions of (a) the lengths of contact with a particular nearest neighbour, (b) the lengths of social contact, i.e. the numbers of successive times a focal individual retained state i*, and (c) the lengths of being alone in the observed data (circles) for the treatment in clear water. Also shown are the means (x's) and the 2.5% and 97.5% percentiles as predicted by our Markov chain models. The graphs (d), (e), and (f) show the corresponding data for the treatment in turbid water. (Note that 0 values cannot be displayed in a logarithmic plot and are omitted.)*

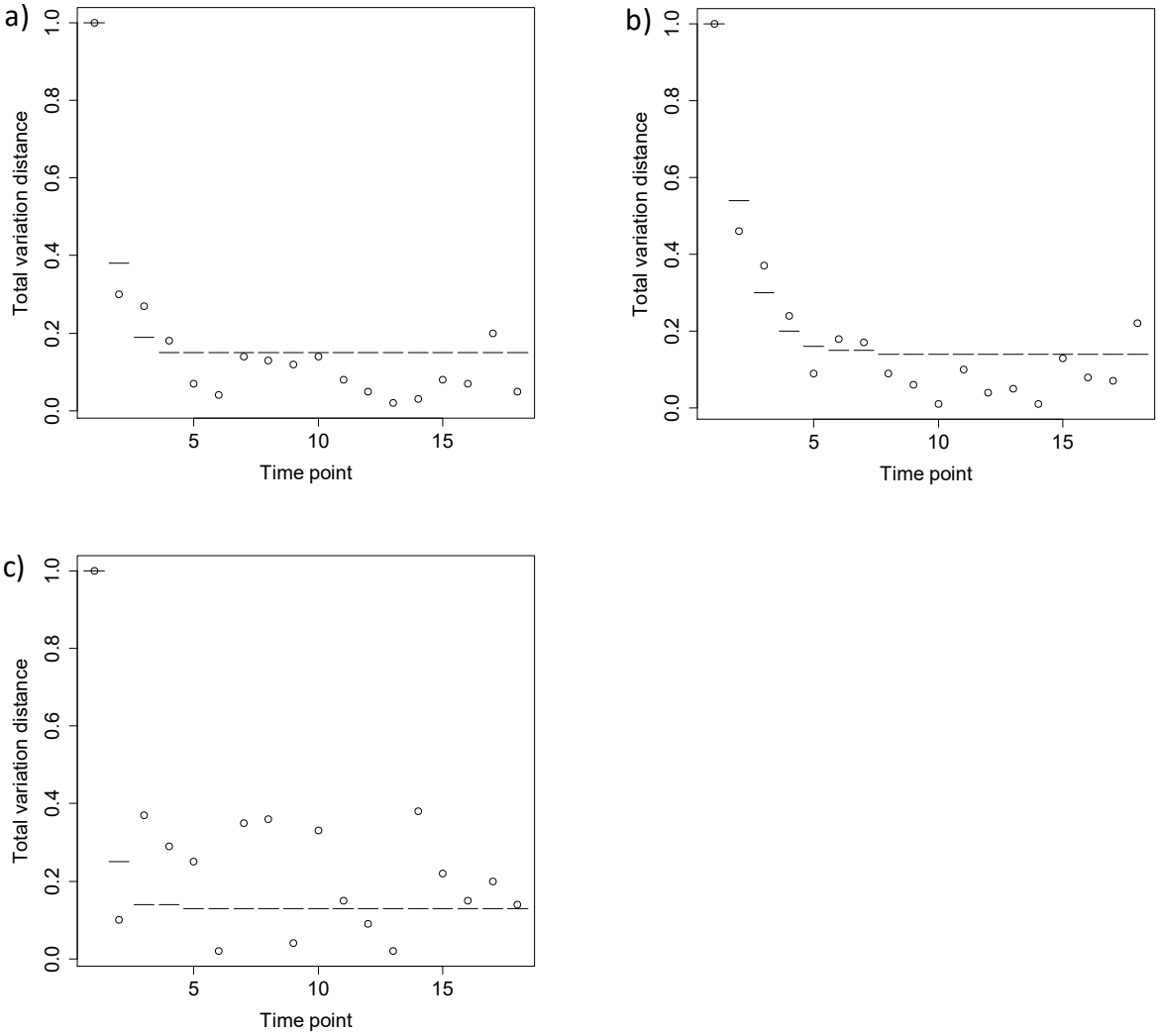

Figure 5 Distance of the frequency distribution of states i *and* x *for the initial state* i *from the frequency distribution of these states for the initial state* x *as a function of elapsed time in the observed sequences (circles) for (a) clear water, (b) turbid water, and (c) a fictitious observation where the behaviour did not follow a Markov chain. The horizontal bars mark the 95% percentiles of this distance as predicted by the Markov chain model.*

Bayesian Information Criterion (BIC)

Although our simple Markov chain model contains only two parameters, the question arises whether the very simple unconditional model with just one parameter (mentioned in the first section of this supplement) can explain the data as well. To answer this question, we applied the Bayesian Information Criterion (BIC) to our data and found that it clearly favours the Markov chain model. The difference between the BIC for the two models was 172.1 in clear water and 531.2 in turbid water. A difference of 9.2 or more is commonly regarded as sufficient for the conclusion that the favoured model is substantially better (Guttorp 1995).

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Chapter II Turbidity-induced changes in shoal structure of guppies (*Poecilia reticulata*) reflect origin-specific experience with turbidity

Submitted manuscript

Karoline K. Borner, Jens Krause, Indar W. Ramnarine, and Thomas Mehner

Turbidity-induced changes in shoal structure of guppies (*Poecilia reticulata*) reflect origin-specific experience with turbidity

Karoline K. Borner[1,2], Jens Krause[1,2], Indar W. Ramnarine,[3] and Thomas Mehner[1]

[1]Leibniz-Institute of Freshwater Ecology and Inland Fisheries Müggelseedamm 310, 12587 Berlin, Germany

[2]Humboldt Universität zu Berlin, Faculty of Life Sciences, Invalidenstraße 42, 10115 Berlin, Germany

[3]Department of Life Sciences, The University of the West Indies, St Augustine, Trinidad & Tobago

Abstract

Social associations of fish which rely on the visual sense may be strongly impaired if transparency of water declines. This may influence benefits gained through social associations such as information flow about food patches or predator threat. In the native distribution range of Trinidadian guppies (*Poecilia reticulata*), quarrying has induced repeated events of enhanced turbidity at stream sites below quarries. We experimentally compared the social interactions of guppies in response to turbidity between fish from below quarries, which experience turbidity regularly (experienced fish), and fish from sites above the quarries (inexperienced fish), where water remains clear. We performed focal fish observations in three consecutive treatments (1) clear water, (2) short-term turbidity and (3) long-term turbidity (four hours) in the laboratory and recorded five variables representing the intensity of social behaviour. While experienced fish initiated fewer associations but increased group size, inexperienced fish kept the number of initiated associations stable but at the cost of lowered group size. Further, inexperienced fish tended to be alone more often and showed fewer mating attempts under turbid conditions than experienced fish. Activity decreases in fish from both origins but even more so in experienced fish. We suggest that experienced guppies stabilized group connectivity under turbid conditions, whereas inexperienced fish decreased group connectivity. Lower connectivity may impair information flow and hence affect benefits of group living which may influence population dynamics. This becomes relevant to conservation efforts because quarrying is still increasing in Trinidad.

Keywords: social behaviour, turbidity, interaction pattern, shoaling

Introduction

Fish are strikingly social organisms; about one quarter of all fish species shoal all their lives, and about one half of fish species shoal for part of their lives (Shaw 1978, Hoare and Krause 2003). Different factors govern the assembly of individual fish (Pitcher and Parrish 1993). In response to a predator threat, shoaling is a beneficial reaction facilitating increased anti-predator vigilance, predator confusion or risk dilution (Krause and Ruxton 2002, Krause et al. 2011). Furthermore, shoaling increases the chance of finding profitable

food patches (Pitcher et al. 1982, Magurran and Pitcher 1983, Vollset and Bailey 2011) and facilitates social learning (Laland and Williams 1997, Lachlan et al. 1998, Webster and Laland 2011).

Fish can gather information directly via personal sampling or indirectly via other individuals. Personal information sampling might be risky, as in the case of a predator threat, or energetically costly, if the sampling area is large. To acquire information from other individuals is less risky and energetically less costly, but the information might be wrong or outdated. By shoaling, information can be visually transmitted when individual fish observe active signalling or reactions of shoal mates to external stimuli (Pitcher and Parrish 1993, Hoare and Krause 2003, Webster and Laland 2011). Visual communication serves for short, middle and long-range information transmission (Guthrie and Muntz 1993, Hawkins 1993) while the lateral line plays a role in short-range communication (Pitcher et al. 1976). Communication via the lateral line within a few centimetres facilitates individual fish to estimate velocity, distance and orientation of surrounding fish but also provides information about startle responses (Partridge and Pitcher 1980, Bleckmann 1993).

Many fish species, the Trinidadian guppy (*Poecilia reticulata*) included, rely primarily on their visual sense for communication (Guthrie and Muntz 1993, Houde 1997). The visual sense is strongly involved in kin recognition and familiarity (Griffiths and Magurran 1999), courtship and shoaling (Chapman et al. 2009), predator inspection (Dugatkin 1997) and foraging (Laland and Williams 1997, Day et al. 2001, Griffiths and Ward 2006). Therefore, changes in environmental conditions which impair the visual sense can modify social interactions and communication patterns of fish populations (Sih et al. 2011) by impacting signal transfer with a potential long-term effect on survival and fitness (Engström-Öst and Candolin 2007, Heuschele et al. 2009, Sluijs et al. 2010). For example, water turbidity can disrupt (Kirk 1994, Bradbury and Vehrencamp 1998, Sluijs et al. 2010) communication signals and can lead to a change in the social interaction pattern. In lab-reared guppy shoals, fish responded to a single turbidity event by staying alone longer or in the same but smaller groups (Borner et al. 2015). Females of the poeciliid fish *Xiphophorus helleri* preferred groups of spotted females to groups of non-spotted females in turbid water compared to clear water (Franck et al. 2001). Furthermore, guppies or sticklebacks (*Gasterosteus aculeatus*) were found to switch to another sense, for example to olfaction, in response to visual impairment to maintain social interactions (Chapman et al. 2010, Heuschele et al. 2009).

In the Northern Range of Trinidad, some populations of the Trinidadian guppy are regularly exposed to turbidity with up to 1000 nephelometric turbidity units (NTU) (KKB and RC, pers. obs.). Quarries have been established in the area recently. This has changed the natural conditions in the streams below by increasing deforestation, currents and erosion (KKB, pers. obs., Agard et al. 2004). With every rainfall, clay of the quarries is washed into the rivers, which increases turbidity and influences the vision of fish (Guthrie and Muntz 1993, Utne-Palm 2002). Clay particles suspended in the water column scatter the light and can shift the spectral composition of the light environment (Utne-Palm 2002, Sluijs et al. 2010, Kelley et al. 2012). In contrast, water remains clear above those quarries, where the rivers are still in their natural states. Therefore, guppies originating from above and below the quarry in the same river experience different environmental conditions during their lifetimes.

In this study, we experimentally investigated the social behaviour of guppies with differing origins, therefore either experienced or inexperienced with turbidity, to understand how their association patterns are influenced by previous turbidity experience. We hypothesized that the fish's prior experience with turbidity is associated with behavioural differences. To cope with turbidity, experienced fish should maintain their shoal size to keep shoaling benefits. In contrast, inexperienced fish should display a decrease of social contacts when exposed to turbid water conditions, as they had no prior experience with how to cope with this environmental change. To elucidate the full potential range of response in social interactions, the experiment did not only observe the immediate response to the onset of turbidity, but also covered modifications of social interactions when fish were exposed to turbidity over several hours.

Methods

Subjects

We collected fish from the Arima River in the Northern Range of Trinidad in July, 2012. They were obtained from different pools of two different origins situated above quarry (origin 1, N 10° 42.143', WO 61° 17.742') and below a number of quarries (origin 2, N 10° 40.747', WO 61° 17.371'). Fish from below quarries are known to be exposed to turbid water conditions regularly after rainfalls, which transport sediment from the quarries into the river. Fish originating from above quarries do not experience turbidity regularly. Fish

were kept in the laboratory in aerated tanks at a temperature of 24°C and a 12/12h day/night (artificial and natural illumination) rhythm to acclimatize. They were fed twice per day with Tetramin dry food.

Experimental setup

Experiments were conducted at The University of the West Indies, St. Augustine, Trinidad in July 2012. From each origin (above and below the quarry), we established ten groups of ten fish (five males, five females). All fish of one group originated from the same pool and were assumed to be familiar with each other. Fish were marked individually with Visible Implant Elastomer Tags (VIE), which are known to have no impact on fish behaviour (Croft and Krause 2004), one day after capture. After tagging, fish were allowed to acclimatize for another two days before experiments started. We conducted observations in test tanks (70 x 70 cm) placed on white polystyrene sheets covered with a grid of 10 x 10 cm to allow the recording of two-dimensional positions of the observed fish. Water depth during observation was 2 cm to ensure recognition of the individual fish from above even in turbid water. Earlier observations have confirmed that guppies often enter very shallow waters under natural conditions (KKB and JK pers. obs.); therefore, such shallow water does not provide unusual conditions for them.

Each group was observed in three different treatments; (1) clear water, (2) recently clouded water (subsequently referred to as 'short-term turbidity') and (3) after 4 hours staying in turbid water (referred to as 'long-term turbidity'). We created turbidity with 2 grams of Kaolin (Amberger Kaolinwerke, Germany) dissolved in 50 ml of water and poured gently into the test tank. Visibility changed from 8-10 NTU in clear water treatment to 800 NTU in short-term turbidity treatment and to about 300 NTU after the third observation in long-term turbidity. Fish were gently transferred from their holding tank into the test tank and were allowed to acclimatize overnight. First observation started approximately 30 minutes after feeding, between 8 and 9 am and was conducted in clear water. About two hours after the first observation, the water in the test tank was clouded as described above and the second observation was conducted immediately afterwards. The final observation took place after another four hours in turbidity. Before each observation, we stirred the water gently with a standardized procedure to stir up the clay or, under clear water conditions, to control for disturbance.

Data collection

We conducted focal follow observations for each fish per group in a randomized order, observing for 2 minutes per treatment. Data were collected every ten seconds, resulting in 13 data points per fish in a given treatment. We recorded all group members of the focal fish and its location relative to the square grid on the bottom of the test tank. As group members, we defined all fish that were within four body lengths of the focal fish. In addition, we counted the number of mating attempts conducted or received by the focal fish over a two-minute period. We calculated values for the following five response variables.

1. *Initiated association*: An initiated association was defined as a fish that showed a distinct movement directed towards the focal fish or received a distinct directed movement from the focal fish, and both continued movement together. We recorded how many times such a contact was observed during one observation period.
2. *Local association*: The sum of all fish within four body lengths of the focal fish within one observation period was calculated. Initiated associations were excluded from this calculation to obtain two independent response variables.
3. *Activity*: If a fish changed its grid position (as measured by the squares under the tank) from one 10 s recording to the next, we defined this as a square crossing (regardless of the number of squares moved). Activity is the sum of square crossings the focal fish executed during one observation period (maximum of possible square crossings is 12).
4. *Time alone*: Among the 13 data points for each focal fish we counted how many times a fish was observed alone.
5. *Mating attempts*: The number of mating events an individual initiated and received within an observation period.

Statistical analysis

Initially, we tested for correlations between the response variables by Spearman's rank correlation tests. All Spearman's r-values were $<|0.6|$ except the correlation between time alone and local association (r_S= -0.76), and hence we decided to run separate tests on all response variables. We used R (R Core Team 2013) and the *lme4* package (Bates et al. 2013) to perform a generalized linear mixed effect analysis fitted by maximum likelihood ("glmerMod") with the response variables activity, time alone, initiated and local associa-

tions and mating attempts. We chose origin (experienced and inexperienced fish) and treatment (clear water, short-term – and long-term turbidity) as fixed effects in our model, because they constituted our planned procedures. Further, we included the interaction between origin and treatment as fixed effect, as we hypothesized that fish with different experience with turbidity should react differently to the treatments. We also entered sex as fixed effect because our earlier studies showed that males and females behave differently (Magurran 2005, Lucon-Xiccato and Bisazza 2014, Borner et al. 2015). As a random effect, we used random intercepts for groups with individuals nested in group. Distribution family was "Poisson". We calculated our "full" model as explained above and gradually excluded all factors that were not significant. The hierarchical models were compared by ANOVA and the Akaike Information Criterion (AIC). If two models did not differ significantly and their AIC was similar, we chose the simpler model. Post-hoc analyses were conducted with pairwise t-tests with p-value adjustment of Benjamini/Hochberg. Graphs were produced with R (Wickham 2009, Baptiste 2012).

Results

Overall, for all five response variables, the factor origin, by itself, was not significant, whereas the interaction between origin and treatment was almost always significant (Table 3). The factor treatment was significant in more than half of the cases.

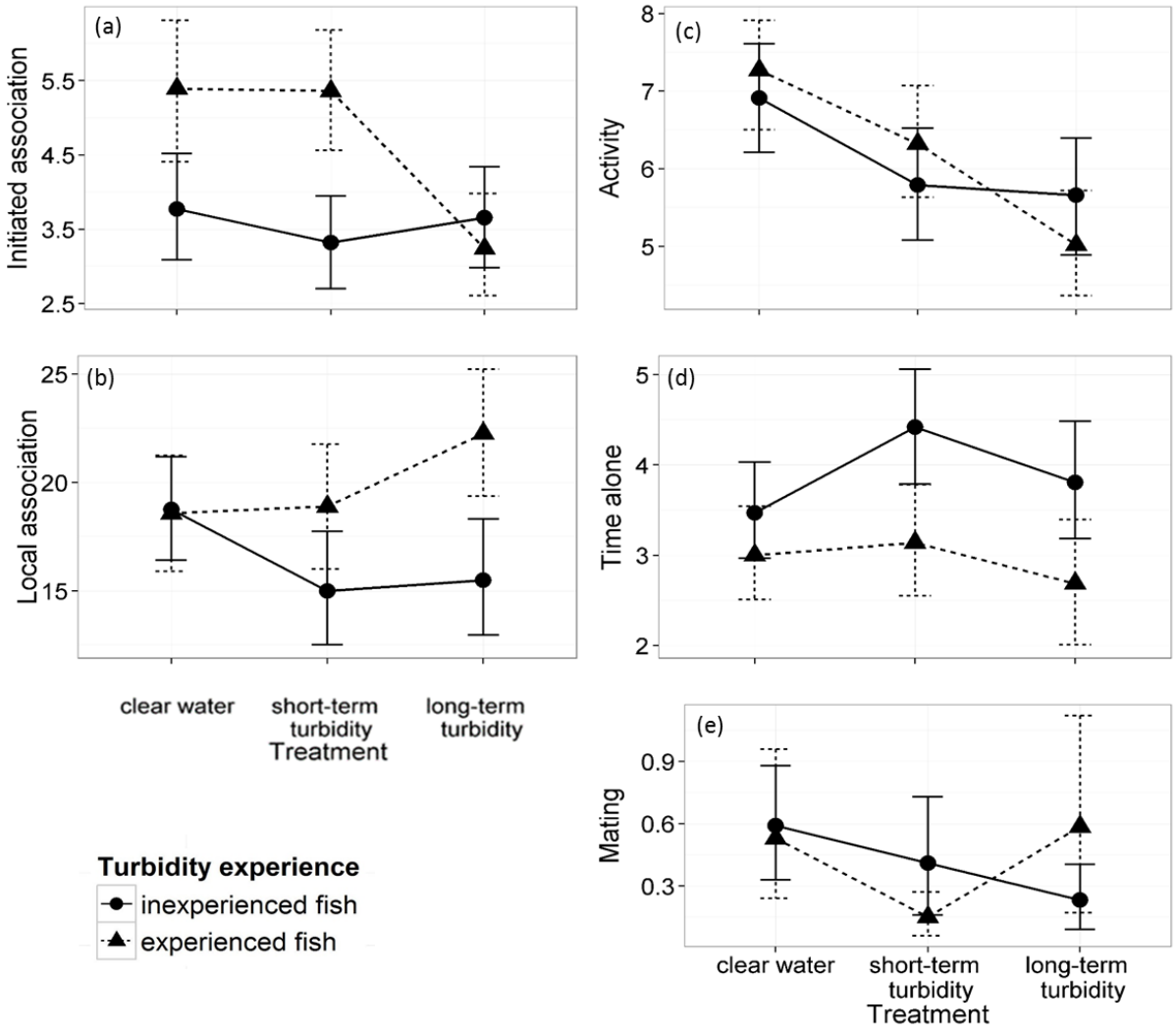

Figure 6 Response variables of social interactions in 10 groups of 10 fish of experienced and inexperienced fish. Continuous lines and circles represent inexperienced fish; dotted lines and triangles represent experienced fish. a) Mean number of initiated associations of inexperienced and experienced fish. b) Mean number of local associations of inexperienced and experienced fish. c) Activity as number of mean square crossings of inexperienced and experienced fish. d) Mean number of time spent alone of inexperienced and experienced fish. e) Mean number of mating attempts given or received of inexperienced and experienced fish. Error bars displays 95% confidence intervals based on bootstrapping. For details of the statistical models, see Table 3.

Initiated association

The fixed factor sex and the interaction term of origin and treatment predicted initiated association significantly (Table 3). Figure 6a shows that experienced fish decreased their number of initiated associations in long-term turbidity significantly (post hoc, p=0.004) in contrast to inexperienced fish. Males had more initiated associations than females (Table 3).

Local association

Treatment, sex and the interaction term of origin and treatment significantly predicted local association. The numbers of local associations decreased in short-term and long-term turbidity (Table 3). Figure 6b shows that inexperienced fish decreased local associations in short-term, whereas experienced fish increased local associations in long-term turbidity (post hoc, p=0.023). Females had significantly more local associations than males (Table 3).

Activity

Activity is significantly predicted by the fixed factors: treatment; sex; and the interaction term of origin and treatment. Activity decreased in the treatments of short-term and long-term turbidity (Table 3, Figure 6c). Females were less active than males in all treatments (Table 3). Post hoc analysis revealed that experienced fish decreased activity significantly from the short-term to the long-term turbidity treatment ($p = 0.009$), whereas inexperienced fish decreased activity significantly from clear water to short-term turbidity ($p = 0.019$) (Figure 6c).

Time alone

Treatment and sex as fixed effects predicted time alone significantly. The interaction term was only marginally significant (in short-term turbidity, p=0.07 and in long-term turbidity, p=0.083), but overall the model with interaction was superior to all other models with fewer terms (Table 3, Figure 6d). Therefore, we kept the interaction in the model and included the results in our discussion. Females spent less time alone than males in all treatments (Table 3).

Mating attempts

Treatment and the interaction term of origin and treatment were significant in our model for mating attempts (Table 3). Inexperienced fish gradually decreased the number of mating attempts over short-term and long-term turbidity. Experienced fish, in contrast, re-established the clear water value for number of mating attempts in long-term turbidity after a temporary decrease in short-term turbidity (Figure 6e).

Table 3 Results of generalized linear mixed model of the response variables activity, time alone, initiated association, local association and mating attempts in response to fish turbidity experience (origin), treatment (clear water, short-term t. = short-term turbidity, long-term t. = long-term turbidity), the interaction origin and treatment, and sex of fish. Reference levels are inexperienced fish, clear water and female.

Response variable	Estimate	Std. Error	z-value	p-value
Initiated association				
intercept	0.888	0.18503	4.799	<0.001
experienced fish	0.39462	0.2522	1.565	0.117
short-term t.	-0.12706	0.07531	-1.687	0.091
long-term t.	-0.03548	0.07366	-0.482	0.631
male	0.36843	0.08439	4.366	<0.001
experienced fish : short-term t.	0.12147	0.09693	1.253	0.210
experienced fish: long-term t.	-0.46619	0.10204	-4.569	<0.001
Local association				
intercept	2.87969	0.11374	25.317	<0.001
experienced fish	0.0124	0.15462	0.08	0.936
short-term t.	-0.22315	0.03464	-6.441	<0.001
long-term t.	-0.19709	0.03444	-5.722	<0.001
male	-0.1967	0.06262	-3.141	0.002
experienced fish : short-term t.	0.23969	0.04762	5.033	<0.001
experienced fish: long-term t.	0.37245	0.04668	7.979	<0.001
Activity				
intercept	1.75355	0.11782	14.884	<0.001
experienced fish	0.04709	0.16149	0.292	0.771
short-term turbidity	-0.17683	0.05637	-3.137	0.002
long-term turbidity	-0.20253	0.05691	-3.559	0.001
male	0.17569	0.05514	3.186	0.002
experienced fish : short-term t.	0.03679	0.07834	0.47	0.639
experienced fish: long-term t.	-0.16334	0.08146	-2.005	0.045
Time alone				
intercept	0.96607	0.10129	9.537	0.001
experienced fish	-0.17612	0.13175	-1.337	0.182

short-term t.	0.24185	0.07189	3.364	0.001
long-term t.	0.09535	0.07463	1.278	0.202
male	0.31696	0.08328	3.806	0.001
experienced fish : short-term t.	-0.19612	0.10831	-1.811	0.070
experienced fish: long-term t.	-0.19535	0.11286	-1.731	0.083
Mating attempts				
intercept	-2.2703	0.52	-4.366	<0.001
experienced fish	0.2502	0.72	0.348	0.728
short-term t.	-0.364	0.2051	-1.775	0.076
long-term t.	-0.9219	0.2483	-3.713	<0.001
experienced fish : short-term t.	-0.8983	0.3591	-2.501	0.012
experienced fish: long-term t.	1.0207	0.3136	3.254	0.001

Discussion

Our results confirm the hypothesis that fish with different turbidity experience respond differently to a turbidity event. Although we found no overall constant modification of the association pattern of fish with different turbidity experience, we found differences in their responses to the three consecutive treatments. Turbidity-experienced fish responded to long-term turbidity with less activity, fewer initiated associations and more frequent local associations than inexperienced fish. Furthermore, inexperienced fish spent more time alone and decreased the number of mating attempts in long-term turbidity compared to experienced fish. These results suggest that experienced fish developed a long-term strategy to cope with regularly occurring turbidity, while inexperienced fish experienced turbidity as a constraint.

The behaviour of fish in their social groups did not differ generally between the two different origins. In particular, the association pattern under clear-water conditions was similar. Fish which originated from below the quarry, are exposed regularly to turbidity (Miliša et al. 2010, Maya et al. 2012), whereas fish from above the quarry live in natural, clear water habitats. The similar behaviour of fish with different turbidity experience in clear water suggests that fish have a preferred social association pattern well-adjusted to clear water conditions that facilitates information transmission, anti-predator behaviour and foraging advantages (Pitcher and Parrish 1993, Hoare and Krause 2003, Krause et al. 2011).

Since turbidity does not persist for longer periods in the natural habitats, a complete switch to another association pattern may not be useful. This conclusion is supported by a study conducted with African cichlids (*Pseudocrenilabrus multicolor*) where only fish reared in permanently turbid water changed their overall behaviour and showed less social behaviours in clear water than fish reared in permanently clear water (Gray et al. 2012). Accordingly, only permanent exposure to turbidity seems to result in enduring differences of the social structure of fish, which could be a heritable trait modification.

Independent of origin, the treatment strongly affected the behavioural response of the fish. Turbidity induced fundamental changes in the social interactions, indicating that the shoal structure expressed in clear water was no longer adequate. However, the significant interaction terms of the GLMM models elucidate that fish with different experience with turbidity responded differently to the three different treatments. Whereas inexperienced fish tended to be alone more often in short-term turbidity, experienced fish did not change time alone between clear water and short-term turbidity. Accordingly, inexperienced fish were separated more often than experienced fish. Separated individuals that rarely interact with others might have fewer opportunities for information exchange and social learning (Couzin et al. 2006, Webster and Laland 2012) about food patches (Laland and Williams 1997) and predator threat (Kristensen and Closs 2004) and hence depend mainly on privately gathered information. This may be costly in terms of personal risk or energy expenditure (Hoare and Krause 2003, Webster and Laland 2011), particularly under turbid water conditions (Abrahams and Kattenfeld 1997). In contrast, experienced fish avoided individual separation. This suggests that avoidance of becoming separated from other fish is an adaptation to turbidity. Also, experienced fish built shoals with higher number of associations, whereas inexperienced fish responded in the opposite way by weakening shoaling.

These results indicate a stronger connectivity in experienced fish in turbid water suggesting that connectivity is the crucial mechanism for social interactions in coping with turbidity. By strengthening the connectivity, fish might compensate for the impairment induced by reduced visibility. They may regain social benefits, such as information transfer (Laland and Williams 1997, Lachlan et al. 1998, Webster and Laland 2011), which strongly concurs with prolonged foraging success and reduced predation risk within a shoal (Pitcher and Parrish 1993, Krause and Ruxton 2002, Kelley and Magurran 2006). The re-established

number of mating attempts by experienced compared to inexperienced fish in long-term turbidity supports this idea.

Stronger connectivity could lead to better adjustment to turbid water conditions in various ways. Firstly, most fishes have well-developed eyes (Guthrie and Muntz 1993) and therefore, the majority of information is transmitted via visual signalling (Abrahams and Kattenfeld 1997). If long-range vision is impaired under turbid conditions, fish might counterbalance this effect through strengthened shoaling to maintain the benefits of visual communication in a shoal. Secondly, fish might as well switch to another sense to circumvent or reduce impairment. Fish not only communicate via the visual sense but also by olfaction (Hara 1993) and lateral line (Pitcher et al. 1976, Bleckmann 1993). With the lateral line, fish can detect movements only within a few centimetres and therefore can gather essential information from their neighbours under impaired visibility only at reduced distance. The enforced shift to another sense as the main information source might reduce shoaling benefits and information transfer, resulting in a reduced frequency of initiated associations, as we found in the response of experienced fish. To retain some benefits of shoaling and to avoid being separated might be more essential than to express individual preferences through initiated associations. In contrast, the response to turbidity of the inexperienced fish may reflect a behavioural constraint, with fish holding up the number of initiated associations and thus having lower potential to restore shoaling and its benefits under impaired visibility. This is supported by our findings, in that inexperienced fish did not restore the number of mating attempts in long-term turbidity. The reduced frequency of mating behaviour might have long-term effects on survival and fitness or may even induce shifts in the genetic composition of populations (Engström-Öst and Candolin 2007, Heuschele et al. 2009, Sluijs et al. 2010)

With respect to activity, inexperienced fish responded only in short-term turbidity with decreasing activity, whereas experienced fish continuously declined activity over all treatments. Interestingly, opposing results for different species can be found in the literature. Cod larvae (*Gadus morhua*), for example, were more active in clear and turbid but not in intermediately turbid water (Meager and Batty 2007). Activity levels of sticklebacks increased with turbidity (Vollset and Bailey 2011). However, studies with African cichlids (*Pseudocrenilabrus multicolor victoriae)* and pike (*Esox lucius)* support our result of decreased activity under turbid conditions (Engström-Öst and Mattila 2008, Gray et al. 2012). Furthermore, individual sticklebacks switched shoals less often in turbid than in clear wa-

ter, which was interpreted as a decrease of activity (Fischer and Frommen 2013). However, high-turbidity conditions and enduring turbidity as applied in our study were not tested in the aforementioned experiments, and hence comparison of results is difficult. However, turbidity might affect species differently, depending on the intensity of their social interactions. Fishes that generally spend more time alone, might experience turbidity as a benefit and use it as cover, leading to higher activity and foraging rates (Gregory 1993, Gregory and Levings 1998). Highly social fishes, which rely on visually transmitted social information, as the guppy does, on the other hand, may experience turbidity as an obstacle. The low activity of experienced fish in long-term turbidity therefore suggests that less activity in turbid water may stabilize the shoals and the social connectivity. Individuals that are more active might split from the group more easily and might have difficulties finding their shoal members again. Further, lower activity may reduce the distance between the members of the group and hence strengthen the communication via the lateral line.

In summary, we found that inexperienced and experienced fish reacted oppositely to turbidity. While inexperienced fish became less social, experienced fish became even more social in turbid, compared to clear water. We assume that the behavioural adjustment of the experienced fish constitutes long-term benefits as they developed their response to a regularly changing environment over longer periods. We therefore conclude that stronger connectivity of groups is the key function in maintaining social processes as it might provide the required structure for information transfer and therefore decision making in an uncertain and rapidly changing environment (Krause et al. 2010). Based solely on these results, we cannot determine whether the high connectivity in long-term turbidity is particularly beneficial for feeding, predator avoidance or reproduction. Subsequent analysis is needed which address the overall value of connectivity for specific behavioural tasks at high and long-term turbidity.

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Chapter III Experience-dependent behavioural responses to ecological challenges in turbidity

Manuscript

Karoline K. Borner, Thomas Mehner, Indar W. Ramnarine, and Jens Krause

Experience-dependent behavioural responses to ecological challenges in turbidity

Karoline K. Borner, Thomas Mehner, Indar W. Ramnarine, and Jens Krause

Abstract

Some guppy populations (*Poecilia reticulata*) in the Northern Range of Trinidad are strongly affected by regularly occurring turbidity due to quarrying activities, and hence are turbidity-experienced while other populations experience only clear water conditions and are inexperienced with turbidity. Guppies may gain benefits from visually mediated shoaling behaviour that may provide higher foraging efficiency and better predator defence but can also be influenced by poor vision. We studied the influence of earlier exposure to turbidity on guppies' response to a food and a predator stimulus. Turbidity-experienced and inexperienced fish from two different rivers were collected and separated into groups of ten fish. We conducted focal follow observations with one focal fish per group in clear and turbid water. Our results show that experienced fish spent less time near the predator dummy in turbid water compared to inexperienced fish and counterintuitively, experienced fish took longer to detect the predator dummy in turbid water compared to inexperienced fish, implying that they were more cautious. No population differences were found in the food dummy experiment. Our results suggest that turbidity-experienced fish cope better with predation risk in turbidity than inexperienced fish and therefore experienced fish may have higher survival rates under turbid conditions. The ecological implications are discussed.

Keywords: guppy, turbidity, predation, predator-prey interaction, shoaling

Introduction

For more than seven decades, the Trinidadian guppy (*Poecilia reticulata*) has been an important model organism in the field of ecology, evolution, and behavioural studies (Houde

1997). Guppy populations in close geographic proximity can experience very different environmental conditions (Magurran 2005) and, therefore, present perfect study subjects for numerous investigations of evolutionary processes (Templeton and Shriner 2004). These differing conditions are also promising for behavioural ecologists as organisms respond to their environment initially behaviourally (Houde 1997, Tuomainen and Candolin 2011). However, the habitat of the Trinidadian guppy, situated in the Northern Range of Trinidad, is changing dramatically. About 20 years ago, quarries started to be established, which included logging of the tropical rainforest. This led to reduced canopy and higher water temperatures, as well as to open ground, which released silt, clay, soil and nutrients into the rivers with every rainfall making the water turbid (Likens et al. 1970, Agard et al. 2004). Turbidity is the cloudiness of the water. Small solid particles are suspended in the water column and attenuate and scatter the light and may shift its wavelength (Utne-Palm 2002). Turbidity can originate from growth of phytoplankton and can be induced by eutrophication through agriculture or, as in the case of guppy habitat, by suspended solid particles arising from deforestation and mining (Sluijs et al. 2010, Maya et al. 2012). Not all guppy habitats in the Northern Range of Trinidad are affected by turbidity, and even fish from the same stream may experience very different levels of turbidity. Guppies living upstream of quarries still live in natural habitats with clear water of about 7 NTU (Nephelometric Turbidity Unit) and hence are inexperienced with turbidity. Guppies living downstream of the quarries experience high turbidity levels, with up to 1000 NTU regularly (KKB and RC, pers. obs.). Few studies have been conducted on guppies concerning differences in turbidity level in their habitats, and these have focused on the distribution of guppies in different environments (Magurran and Phillip 2001), the courtship behaviour of guppies from turbid lowland and clear mountain streams (Luyten and Liley 1985), sensory plasticity (Chapman et al. 2010) or social dynamics (Borner et al. 2015). However, until now, no serious attempts have been made to understand the influence of the relatively new phenomenon of regularly occurring clay-turbidity on the guppies, which is likely to affect the ecology and evolution of those guppy populations.

Guppies spend much of their time shoaling in highly dynamic social groups (Magurran and Seghers 1991, Croft et al. 2003). Shoaling has been identified to be associated with several adaptive benefits (Krause and Ruxton 2002, Krause et al. 2011), for example predator defence mechanisms such as the confusion effect (Landeau and Terborgh 1986), the encounter dilution effect (Pitcher and Parrish 1993) and the many-eyes-effect (Godin et al.

1988), and also higher foraging efficiency (Day et al. 2001). Neill and Cullen (1974) demonstrated that bigger shoals reduced the probability of a successful strike by a predator. Further, bigger shoals locate food faster than smaller shoals (Pitcher et al. 1982). These benefits are facilitated through social learning and information transfer within the shoal, which may largely rely on visual cues (Laland and Williams 1997, Kelley et al. 2003). By watching their shoal members, fish can gain information about food patches or predator attacks (Webster and Laland 2011). Since many fish have well-developed eyes (Griffiths and Ward 2006), it is proposed that vision is very important for social learning (Pitcher et al. 1976, Guthrie and Muntz 1993, Day et al. 2001) and consequently for the ecological benefits, namely foraging efficiency and predator defence. The interaction of vision and social learning makes them both dependent on water clarity and hence may be affected by turbidity.

Organisms that rely on vision as their main information source can be affected differently by turbidity when facing ecological challenges. This depends on their size (Utne-Palm 2002), foraging behaviour (Zamor and Grossman 2007), anti-predator behaviour (Lehtiniemi et al. 2005, Leahy et al. 2011) and shoaling behaviour (Gray et al. 2014). Small fish that visually detect their prey over short distances are less distracted by turbidity than piscivorous species that detect their prey from a long distance (Utne-Palm 2002). For piscivores, reactive distance to prey is reduced and they may have reduced predation success (Giske et al. 1994, Fiksen et al. 2002). Prey may be impaired in their predator defence mechanisms (Ferrari et al. 2010) and mate choice mechanisms may be interrupted (Seehausen et al. 1997). Some prey organisms use turbidity as cover and reduce their anti-predator behaviour (Gregory and Northcote 1993). Others change their activity level (Vollset and Bailey 2011), mate choice pattern (Candolin et al. 2007), or association behaviour (Heubel and Schlupp 2006). Trinidadian guppies responded to turbidity, depending on their earlier experience, by forming smaller group sizes (inexperienced fish) or larger group sizes (experienced fish) (Borner et al. submitted). Whether such altered behavioural responses help individuals to cope with ecological challenges such as foraging or predation risk in turbidity must be considered separately for each species.

Therefore, we conducted an experiment with guppies from areas located above and below quarries and were thus inexperienced and experienced with turbidity. We tested whether shoals of experienced fish handled ecologically relevant tasks differently in the event of increasing turbidity (as happens regularly after rainfall in their habitats) compared to shoals of inexperienced fish. We chose food and a predator threat as stimuli, as they are

paramount ecological challenges. We observed one focal fish of each group as a representative of the whole group. Fish are generally expected to be attracted by the food and repelled by the predator regardless of origin and turbidity experience. We hypothesized that the ability to perform these responses should be compromised by turbidity. Fish that are experienced with turbidity were predicted to respond to a predator sooner in turbidity and to visit the risky area less often.

Methods

Study species

Fish from two different rivers (Arima and Turure) of the Northern Range of Trinidad were collected in March 2014. From each river, we chose fish from two different origins to provide fish inexperienced with turbidity (above quarry) and experienced with turbidity (below quarry). In the laboratory, fish were kept in aerated tanks at a temperature of 24°C and a 12/12h day/night rhythm to acclimatize. All fish were fed with Tetramin dry food twice a day.

Experimental setup

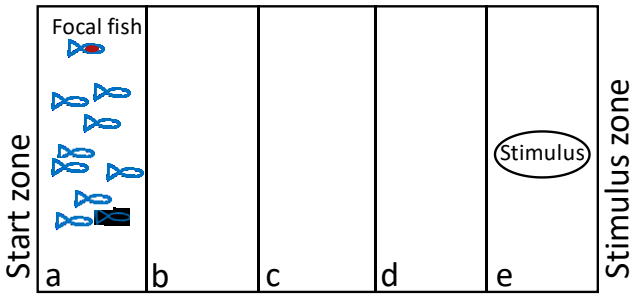

Figure 7 Test tank. Underneath the tank, 5 zones were marked to locate the focal fish. All fish were shifted into the start zone before each trial and the stimulus was set up in the stimulus zone.

Experiments were planned with 100 fish per river and origin. Due to logistical issues, we managed to get only 80 inexperienced fish from the Turure River; hence, we conducted experiments with 380 fish in total. From each location, groups of ten fish (five males, five females) were formed prior to the experiments. Fish of each group originated from the same pool and were assumed to be familiar with each other. The test tank measured 90 x 40 cm, with water height kept at 5 cm. The tank was wrapped in white paper to avoid disturbance from outside the tank. One flank was left open to allow for observation from the side. Underneath the tank, 5 zones were marked as shown in Figure 7 to locate the focal

fish and record its position. We chose two different stimuli – food and a predator – as ecologically relevant factors. To avoid fish receiving information via olfaction, stimuli were chosen to be dummies. The food dummies consisted of 12 pieces of red wire of 1 cm in length and 1 mm in diameter scattered in the stimulus area (Figure 7). These red food dummies mimicked red fruits, which are known to be an important food source that provide carotenoids for Trinidadian guppies. As predator dummy we used a 12 cm long plastic fish (used for fishing gear). Pilot studies confirmed that the food dummy induced attraction and pecking behaviour while the predator dummy induced repulsion and predator inspection behaviour. Turbidity was produced with two grams of Kaolin (Amberger Kaolinwerke, Germany) dissolved in 50 ml of water and poured gently into the test tank. This resulted in a turbidity level of about 330 NTU. Clear water during trials measured about 5 NTU.

All groups were subject to four treatments in a row: (1) clear water and food, (2) clear water and predator, (3) turbid water and food and (4) turbid water and predator. The day before the experiment started, the fish were transferred into the test tank filled with clear aerated water and were left for acclimatisation overnight. Experiments started next day in the morning. Fish were gently shifted to the start zone of the test tank (Figure 7) with a dip net, and the stimulus was set up in the middle of the stimulus zone before the dip net was gently removed. The observer sat motionless next to the tank and began data collection only when the fish started to move naturally. Following the two primary experiments with the food and predator dummies in clear water, the water in the test tank was clouded. Fish were allowed to acclimatize for four hours in turbid water and the food and predator experiments were conducted. We deliberately decided not to randomize the order of the experiments as fish might differ in the way they approach the stimulus zone depending on what they experienced previously, a benefit (food) or a threat (predator). We are aware of the possible presence of bias with fish being more timid in the food experiment, because it was the first experiment conducted. Therefore, we analysed the data of the food and the predator experiments separately and did not compare the absolute values these 2 experiments' results. Further, we kept the order of first clear and then turbid water treatment to represent a treatment similar to the natural occurrence of turbidity.

Data collection

We conducted direct focal fish observations. In each group, one fish was chosen as the focal fish and was observed subsequently in all four treatments. The fish was identified by natural colour marks and size. The focal fish served as an approximate indicator of behaviour for the whole group. As males and females differ in their (social) behaviour (Darden et al. 2009, Lucon-Xiccato and Bisazza 2014, Borner et al. submitted), half of the focal fish chosen were females and the other half were males. In each treatment, the focal fish was observed over a 6-min period and the position of the focal fish in the zones was recorded every ten seconds with "J-watcher" (a software tool for quantitative behaviour analysis). Thus, we obtained 6 x 6 =36 data points (6 min with one data point every 10 seconds). Further, we recorded the time until the first fish of the shoal responded to the stimulus. Response to the stimulus was defined as a fish entering the stimulus zone or showing attraction (food) or repulsion (predator) reaction.

Data analysis

During the 6-min observation period, we observed different phases of behavioural activities. At the beginning, when the dip net was removed, fish began exploring the arena ("exploration phase"). When the stimulus was detected, fish responded – depending on the stimulus – with attraction or repulsion, followed by inspection of the stimulus. This "inspection phase" was surprisingly short and took on average 40 seconds. After the "inspection phase", fish learned that they could gain no benefit from the food dummy or were not threatened by the predator dummy and habituated to them. Once habituated, some fish even started pecking at the predator dummy or ignored the food dummy. Since we aimed to investigate the response of fish to ecologically relevant stimuli, we excluded the data points where fish already showed habituation to the stimuli (habituation phase) and the exploration phase (where the stimulus was yet undetected). Hence, we focused on the data points of the inspection phase, which included four data points as the inspection phase lasted 40 seconds (on average). We obtained a frequency distribution over the five zones of each focal fish in each treatment for these 40 seconds.

We used a generalized linear mixed model (GLMM) fit by maximum likelihood ("glmerMod") with "Poisson" as the distribution family. Response time was the dependent variable with origin (inexperienced and experienced fish), treatment (clear and turbid water), and the interaction of origin and treatment as fixed factors. We analysed the frequen-

cy of stay of the focal fish in the stimulus zone with origin, treatment, and the interaction term of origin and treatment as fixed factors. In both analyses, the random factor was included as random intercepts for group nested in site (i.e., Arima or Turure River). All statistical calculations were conducted in R (R Core Team 2013) and the *lme4* package (Bates et al. 2013).

Results

Response time

We did not find a significant effect in any of the food stimulus experiments (Table 4, Figure 8a).

In the predator stimulus experiment, response time was significantly predicted by the fixed factors treatment and the interaction of origin and treatment. Generally, fish in turbid water took longer to respond to the predator stimulus than fish in clear water (Table 4). Inexperienced fish responded to the predator stimulus faster in turbidity than experienced fish in turbidity (Figure 8b).

Table 4 Response time. Results of the generalized linear mixed model (GLMM) analysis of time until the first fish of the shoal responded to the stimulus (food or predator). Fixed factors were origin (inexperienced and experienced fish), treatment (clear and turbid water) and the interaction of origin and treatment. Random factor was random intercepts for group nested in site (Arima or Turure). Distribution family was "Poisson".

Food	***Estimate***	***Std. error***	***z value***	***p value***
intercept	4.41462	0.19008	23.225	<0.001
experienced fish	0.25268	0.22206	1.138	0.255
turbid water	0.03682	0.04596	0.801	0.423
experienced fish : turbid water	-0.11465	0.05884	-1.948	0.052
Predator				
intercept	3.18559	0.19007	16.76	<0.001
experienced fish	0.26326	0.24972	1.054	0.292
turbid water	0.88301	0.07085	12.464	<0.001
experienced fish : turbid water	-0.19959	0.08459	-2.360	0.018

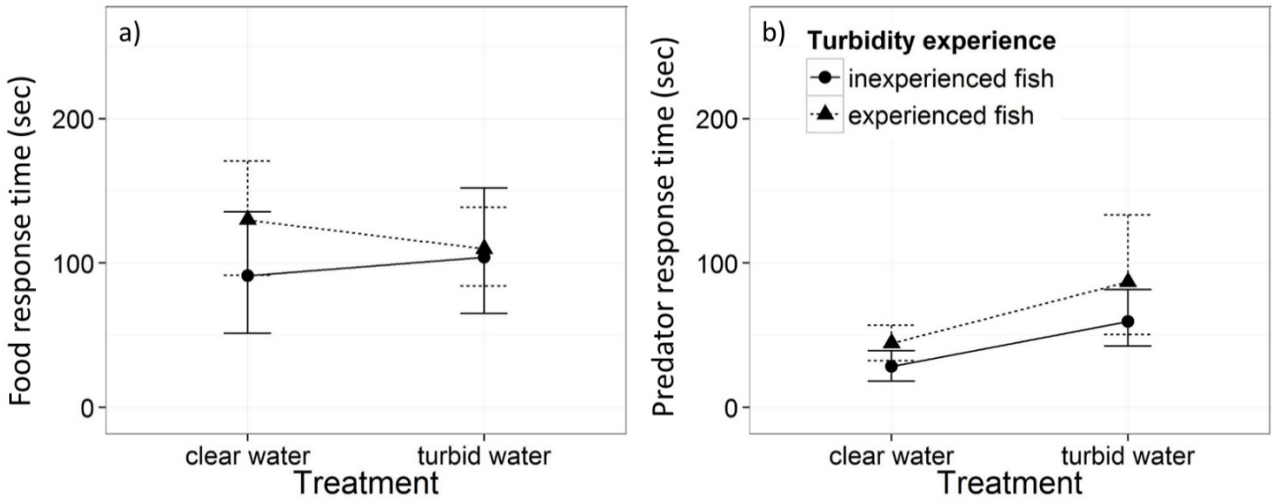

Figure 8 Response time. Interaction of origin and treatment. Graphs illustrating interactions between origin and treatment and show time in seconds until fish responded to stimulus a) food (n=58) and b) predator (n=59). Circle and solid line display data of inexperienced fish, triangle and dashed line display data of experienced fish. Error bars display 95% confidence intervals based on bootstrapping.

Vulnerability time (Frequency of stay in the stimulus zone)

We did not find any significant effects in the food stimulus experiments (Table 5, Figure 9a).

In the predator stimulus experiments, frequency of stay of the focal fish in the stimulus zone was significantly related to the interaction of origin and treatment (Table 5). Figure 9b indicates that experienced fish decreased frequency of stay in the stimulus zone in turbid water while inexperienced fish increased frequency of stay in the stimulus zone.

Table 5 Frequency of stay in stimulus zone. Results of the generalized linear mixed model (GLMM) analysis of the frequency of stay of focal fish in the stimulus zone in food and predator experiment. Fixed factors were origin (inexperienced and experienced fish), treatment (clear and turbid water) and the interaction of origin and treatment. Random factor was random intercepts for group nested in site (Arima or Turure). Distribution family was "Poisson".

Food	***Estimate***	***Std. error***	***z value***	***p value***
intercept	-1.69116	0.68594	-2.466	0.014
experienced fish	1.2432	0.77945	1.595	0.111
turbid water	-0.27789	0.89394	-0.311	0.756
experienced fish:turbid water	0.04626	0.98411	0.047	0.963
Predator				
intercept	-0.2983	0.3397	-0.878	0.38
experienced fish	0.7857	0.4039	1.946	0.052
turbid water	0.445	0.4053	1.098	0.272
experienced fish:turbid water	-1.2612	0.5232	-2.41	0.016

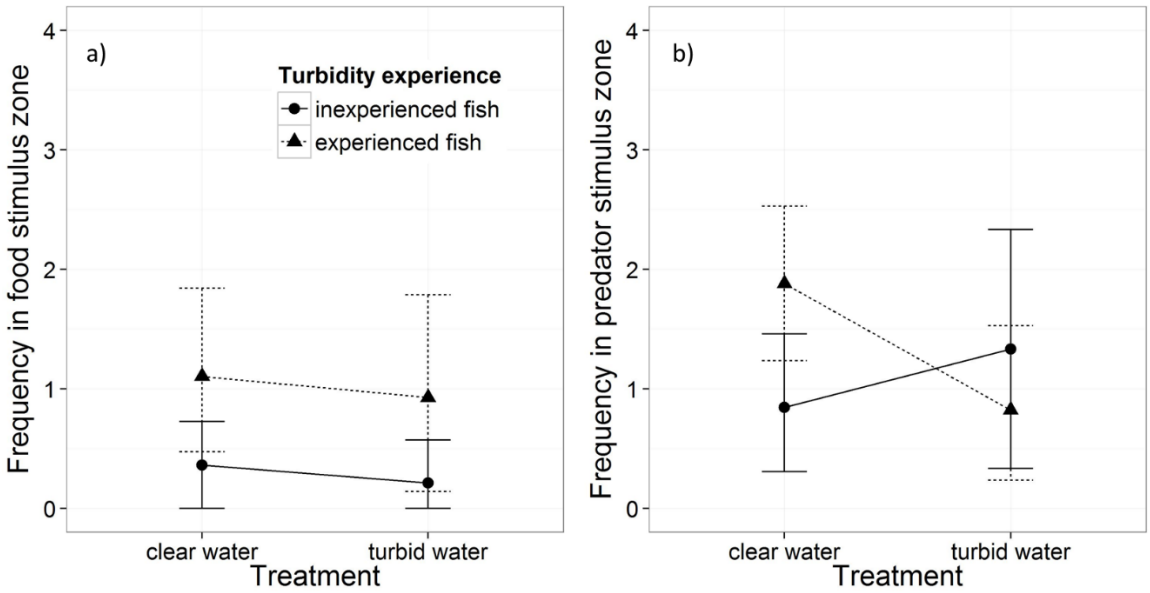

Figure 9 Vulnerability time. Plots illustrating interactions between origin and treatment in the a) food experiment and b) predator experiment. Graphs show frequency of stay of the focal fish observed in the stimulus zone in clear and in turbid water. Circle and solid line display data from inexperienced fish, triangle and dashed line display data from experienced fish. Error bars display 95% confidence intervals based on bootstrapping.

Discussion

Our results partly support our hypotheses and show that fish with differing turbidity-experience respond differently to predation risk but not to a food stimulus in turbid water. All fish regardless of the experience-level with turbidity, took more time to respond to a predator threat in turbid than in clear water. Additionally, turbidity-experienced fish reduced time spent in the predation risk area in turbidity compared to inexperienced fish. Further, they took longer to respond to a dummy predator in turbid water than inexperienced fish. Those findings have potentially important implications for the survival of fish and hence for their future fitness.

Turbidity is known to have direct effects on fish´s visually mediated behaviour such as mate choice (Seehausen et al. 1997, Heuschele et al. 2009), predator prey interactions (Abrahams and Kattenfeld 1997), foraging (Zamor and Grossman 2007) and decision making processes regarding predation rates and foraging efficiency (Lima and Dill 1990). In this study, we found that differing earlier experience with turbidity also strongly impacted fish's visually mediated behaviour and led to different behavioural responses under turbid conditions.

Response time

Turbidity delayed the response to the predator stimulus in experienced and inexperienced fish. Since turbidity particularly impairs long-range vision under high turbidity levels, reactive distance to piscivorous fish is reduced (Vinyard and O'brien 1976, Barrett et al. 1992, Utne-Palm 2002). Hence, guppies needed to get closer to the stimulus by chance while exploring the arena to detect it. Longer response time of experienced fish compared to inexperienced fish in turbid water may suggest that experienced fish were more cautious during exploration when their main information source about their environment – the ability to use visual cues– was impaired. The benefits of anti-predator behaviours such as dilution effect or confusion effect are compromised in turbid water as with reduced reactive distance predation risk mainly depends on who is first detected by the other (Abrahams and Kattenfeld 1997). Therefore, the chance for prey to control predation risk is to reduce encounter rate. Decreased exploration may hence reduce encounter rate with the predator and increase survival probability in turbid water.

The mechanism of the observed response may be that fish reduced their activity level, which increased exploration time. This is supported by the findings in Borner et al. (sub-

mitted), where experienced fish decreased activity in longer lasting turbidity. Decreased activity in turbidity was also found in African cichlid, *Pseudocrenilabrus multicolor victoriae* (Gray et al. 2012) and in sticklebacks (Fischer and Frommen 2013). However, pike larvae, *Esox lucius* and Chinook salmon, *Oncorhynchus tshawytscha*, decreased cover seeking in turbidity (Gregory and Northcote 1993, Lehtiniemi et al. 2005).

Vulnerability time

Turbidity-inexperienced fish were, under turbid conditions, more exposed to the predation risk area than turbidity-experienced fish. This suggests that the probability of fish being attacked and potentially killed by a predator is increased and ultimately reduces survival rate and individual fitness.

The behavioural response of inexperienced guppies may be the result of the constraints imposed on them by turbidity. These constraints may occur on different levels. First, inexperienced fish may have been less aware of the predator stimulus, as long-range vision and reactive distance between predators and prey are reduced under highly turbid conditions, (Giske et al. 1994, Sweka and Hartman 2003). Second, recognition of the predator after detection may be impaired in inexperienced fish as turbidity scatters the light and makes the outline of an object look fuzzy. This may impair the fish to generalize their (in the field) learned predator recognition and hence suffer higher predation risk. This was found in fathead minnows, *Pimephales promelas,* where fish failed to generalize the appearance of brown trout to the appearance of rainbow trout in turbid water and hence omitted anti-predator response (Ferrari et al. 2010). When we collected our test fish in the field, we paid careful attention that collection pools of experienced and inexperienced fish were located as close as possible to each other to make sure all fish had a similar experience with predators such as Crenicichla and Characins.

Third, visual social information transmission within the shoal may be compromised due to poor visual conditions and hence less information about the predator is available within the shoal. Social information is important for learning about predator identities (Manassa and McCormick 2012), implying that a decrease in social information transmission may influence predation risk. In contrast, turbidity-experienced fish showed stronger avoidance of an area with potentially higher predation risk under impaired visual conditions (Figure 9b). The behavioural response may indicate that experienced fish were more cautious and less inquisitive in a little-known environment. A similarly cautious response was found in

turbidity-experienced coho salmon *(Oncorhynchus kisutch)* that reduced any social activities in turbidity (Berg 1983).

Organisms may compensate for reduced vision by increasing their reliance on other senses such as olfaction or lateral line (e.g. threes-pined stickleback, *Gasterosteus aculeatus*, guppy, saithe, *Pollachius virens*) (Pitcher et al. 1976, Webster et al. 2007, Chapman et al. 2010). Guppies are known to recognize a predation threat due to perception of alarm substance through olfaction (Hoare et al. 2004), and may hence rely more strongly on olfaction in turbidity. Therefore, we excluded in our experiment other cues than vision by using a predator dummy in order to exclude such compensation. However, avoidance behaviour may also be connected with information transmission within the shoal. If only one fish gathered information about the existence of a predation threat and this information was transmitted successfully within the shoal, all fish may be able to avoid the risky area. Borner et al. (submitted) found altered social structures in turbidity-experienced fish, which may conceivably be connected to the predator threat response.

Overall, experienced fish seemed to cope better with turbidity concerning predation risk. However, predators may also change their hunting behaviour and become more active to increase encounter rates (Abrahams et al. 2007). In addition, we know from experiments placing guppies in clear bottles in streams that acara cichlids, *Aequidens pulcher*, pike cichlids, *Crenicichla frenata*, and wolf-fish, *Hoplias malabaricus* are attracted at least partly via visual stimuli to their fish prey (Botham et al. 2005). To which extent these predators might also use olfactory cues in turbid water is not known. However, other predators that are known to use olfaction to find prey and that are normally mostly nocturnal such as freshwater prawns, *Macrobrachium carcinus* and catfish, *Rhamdia quelen* (Botham et al. 2008) might become more diurnal in turbid waters. A switch in dial activity patterns of nocturnal predators in response to enhanced turbidity could further affect guppy behavioural response and may have profound effects on the persistence and evolution of the guppy.

Conclusion and perspective

By analysing one focal fish per group as indicator for the whole group, we obtained some conclusive results. However, since we only used four data points for the analysis the outcome should be taken as preliminary results.

We found no differences between the experienced and inexperienced fish regarding the food experiment although we detected a trend in the interaction term ($p=0.052$) of detection time. Indeed the food and predator stimuli covered the same area but the predator stimulus was larger in volume and hence may have had a stronger effect. In addition, test fish were fed twice a day to ensure the same precondition for all fish. Therefore, fish were not food deprived before the experiment and hence foraging was less essential. Hence, the food experiment may be considered as a control as it served as novel object for the fish but in the context of the experiment did not have as great of an ecological impact as the predator treatment. In contrast, a predator threat represents an immediate hazard for survival and should always be considered. However, a trend was observed in the food experiment and with a bigger sample size, it might be possible that we would find statistically significant differences between populations that are also behaviourally substantive. Further, the cohesion within a shoal and the distribution of the fish in subgroups in the different zones might give insights into the mechanisms behind their adjustment to turbidity.

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Declaration of authorship

I hereby declare that this PhD thesis has been written only by the undersigned and that no sources other than those indicated have been used. This thesis has not been submitted for a doctor's degree at any other institution. I am aware of the underlying doctorate regulations of the faculty this thesis is submitted to, i.e., Faculty of Life Sciences of the Humboldt-University at Berlin.

Hiermit erkläre ich, die Dissertation selbständig und ohne unerlaubte Hilfe angefertigt zu haben. Ich habe mich nicht anderwärts als Doktorand beworben. Ich erkläre die Kenntnisnahme der dem Verfahren zugrunde liegenden Promotionsordnung der Landwirtschaftlich-Gärtnerischen Fakultät der Humboldt-Universität zu Berlin.

Berlin, den 08.06.2015

Karoline Borner

Leibniz-Institute of Freshwater Ecology and Inland Fisheries

Forschungsverbund Berlin e.V.

Humboldt-Universität zu Berlin

Faculty of Life Sciences